UNIVERSITY OF ALBERTA

DIFFERENTIAL RESPONSES OF WHEAT CULTIVARS (*TRITICUM TURGIDUM* L. VAR. *DURUM* AND *T. AESTIVUM* L.) TO DROUGHT STRESS

BY

JOSEPH MOGIRE NYACHIRO ©

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in PLANT BREEDING**

DEPARTMENT OF AGRICULTURAL, FOOD AND NUTRITIONAL SCIENCE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **DIFFERENTIAL RESPONSES OF WHEAT CULTIVARS (*TRITICUM TURGIDUM* L. VAR. *DURUM* AND *T. AESTIVUM*) TO DROUGHT STRESS** submitted by **J. MOGIRE NYACHIRO** in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **PLANT BREEDING**.

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"In sub-Saharan Africa, one of the first wheat breeding programmes was established in 1906 in Kenya by a wealthy settler, Lord Delemere. Delemere recruited a British plant breeder, G.W. Evans, to develop rust resistant varieties by crossing varieties of Australian, Canadian, Egyptian, and Italian origin. Evans and his successors found that only a few of the imported varieties could be recommended for release without further breeding and selection. The breeding programme was eventually taken over by the colonial government, which established the International Rust Station now National Plant Breeding Research Centre, Njoro, the main wheat research centre in Kenya"
(Gebre-Mariam, Tanner and Hulluka, 1991).

ABSTRACT

The aim of this study was to determine the utility of commonly measured traits in studies of drought and drought tolerance in predicting drought tolerance. Cultivar tolerance was defined using the drought susceptibility index (DSI) (Fischer and Maurer, 1978), where relatively low DSI values indicate high tolerance.

Growth room studies were conducted using a split-plot design comprising three replicates, two watering treatments (non-drought and drought stressed) as the mainplots, and eight wheat (two tetraploid and six hexaploid) cultivars as the subplots. Drought was induced when plants were at tillering growth stage. Leaf relative water content, electrolyte leakage (EL), leaf chlorophyll content (Chl), photosynthetic efficiency, non-structural carbohydrates, free amino acids, total protein content, nitrate reductase activity, grain yield, yield components, and several morphological traits were assayed to test which of these can predict DSI.

Results showed that cultivar DSI values varied from 0.54 to 1.29. Predictability of DSI was not improved significantly by use of multi-variables. Differential significant drought response of the cultivars was common, but only EL and total Chl content predicted DSI with a large R^2 (> 0.67). DSI and EL were negatively correlated (r = - 0.77) under drought conditions, indicating that drought tolerant cultivars can be identified by measurement of EL. Under non-drought conditions, Chl *a* and total Chl were positively correlated (r > 0.77) with DSI, indicating that drought tolerant cultivars were associated with low Chl content. Under drought conditions, high correlations to DSI (r = - 0.84) were found for kernels spike^{-1} and plant height. Also, classification of these cultivars for drought tolerance could be made in the absence of drought, using a measure of Chl content, or following drought stress, by measuring EL, kernels spike^{-1} and plant height. From a breeder's perspective, the use of EL or Chl would be more convenient.

DEDICATION

This thesis is dedicated to my living parents **S. Mogire Kebati** and **J. Nyanchera Mogire** for denying themselves many things in life for the sake of my education foundation. Dedication is also extended to the entire born and unborn family of **Mogire** for their inspiration.

PREFACE

Plant breeding, the science of crop improvement, has played a significant role in developing wheat cultivars that can grow well under a wide range of environments. This has been achieved through breeding for agronomic traits that can contribute to high grain yield. However, it is still not fully understood how traits directly or indirectly influence grain yield. Nor is it fully understood how various agronomic traits interact with other traits and various environments, such as drought stress, in determining grain yield.

It is believed that improved wheat cultivars will photosynthesise more efficiently and convert a greater portion of biomass to grain yield. Further, it is believed that to improve wheat cultivars a knowledge of the appropriate agronomic traits, especially under drought conditions, is important. Techniques complementing classical plant breeding methods would provide the means and knowledge upon which to draw up the specifications for cultivar selection.

This thesis comprises five chapters focusing on differential responses of wheat cultivars to drought. Chapter 1 covers the literature review pertinent to the topic. Chapters 2 to 4 describe the experiments that were conducted to evaluate the effects of drought on wheat cultivars, and to determine the traits that could predict drought tolerance. Chapter 5 covers the general discussion and important conclusions of the study.

Disclaimers: Mention of commercial products or trade names in this thesis does not constitute endorsement or recommendation by the author, the Examining Committee, the Department of Agricultural, Food and Nutritional Science at the University of Alberta, or the Kenya Agricultural Research Institute. The author assumes responsibility for any errors and omissions that may arise in this thesis.

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LIST OF ABBREVIATIONS

µg	Microgram
µL	Micro-liter
ANOVA	Analysis of variance
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
Ch T	Total chlorophyll
Chl *a*	Chlorophyll *a*
Chl *b*	Chlorophyll *b*
CIMMYT	Centro Internacional de Mejoramiento de Maiz y Trigo
CPS	Canada Prairie Spring
cv.	Cultivar
DAP	Days after planting
df	Degrees of freedom
DSI	Drought susceptibility index
DTT	Dithiothreitol
DW	Dry weight
EC	Electrical conductivity
ED	Early drought
EL	Electrolyte leakage
FAO	Food and Agricultural Organisation
Fm	Maximal fluorescence
Fo	Non-variable fluorescence
Fv	Variable fluorescence
Fv/Fm	Photochemical efficiency of photosystem II
FW	Fresh weight
g	Force of gravity
HPLC	High-performance liquid chromatography
ICARDA	International Centre for Agricultural Research in the Dry Areas
LD	Late drought
LSD	Least significant difference
mg	Milligram
mL	Milliliter
mM	Milli-molar
MPa	Megapascal
NADH	Nicotinamide adenine dinucleotide reduced form
NaF	Sodium fluoride
nm	Nanometer
NR	Nitrate reductase
NRA	Nitrate reductase activity

NSC	Non-structural carbohydrates
°C	Degree Celsius
PMSF	Phenylmethylsulfonyl fluoride
r	Simple linear correlation coefficient
R^2	Coefficient of determination
RMD	Residual moisture drought
RWC	Relative water content
SAS	Statistical analysis system
SDS	Sodium dodecylsulfate
SE	Standard error
TKW	Thousand-kernel weight (g)
TW	Turgid weight
v/v	Volume/volume
Var.	Variety
w/v	Weight/volume
WSC	Water soluble carbohydrates
YFEEL	Youngest fully emerged and expanded leaf

CHAPTER 1
LITERATURE REVIEW

1.1 INTRODUCTION

Wheat (*Triticum aestivum* L. and *T. durum*), one of the most widely cultivated cereal crops in the world, is believed to have originated somewhere in the Middle East, possibly in the area around the Euphrates-Tigris river basin (Braidwood, 1958). Deliberate cross-breeding of crops began about 170 years ago, although conscious selection of robust plants was practiced much earlier (CIMMYT, 1993).

1.1.1 Global Wheat Situation

Wheat occupies over 25% of the world's total area devoted to cereal grain cultivation. In 1996, 570 million t of wheat were forecast to be produced on 220 million ha worldwide (FAO, 1996). According to CIMMYT (1995), developing countries produce 43% of the global wheat with an average annual growth rate of about 4.1% compared to 2.2% in industrialised countries. Between 1961 and 1990, wheat consumption in developing countries grew at a rate of 4.6% per year compared to 3.4% for all other cereals. The global wheat consumption was estimated at 555 million t yr^{-1} for the years 1992-93, at which time it was predicted that the worldwide demand for wheat would grow at 3.0% yr^{-1}. In 1995, for example, on a regional basis, the sub-Saharan Africa demand for wheat was estimated to grow at 3.3% yr^{-1} compared with a demand of 2.5% yr^{-1} in industrialised countries (CIMMYT, 1995). By the year 2005, developing countries will consume approximately 60% of the world's total wheat stocks, compared with their current consumption of 50% (CIMMYT, 1995). This relatively high demand for wheat will require more research directed to making the best of local environmental conditions. Thus, the development of wheat cultivars that can tolerate local environmental conditions, such as drought, must be incorporated and encouraged in wheat improvement programmes.

Drought and wheat in Kenya. In Kenya, wheat is produced in small-scale holdings, ranging between 1.4 and 2.3 ha, with minimal or no mechanisation, and large-scale mechanised farms with large tracts of land. Wheat ranks second after maize as the staple cereal crop of Kenya with an annual domestic production of about 252,000 t. To meet domestic requirements, 262,000 t have to be imported annually (FAO, 1996). In Kenya, approximately 66% of the total land area is semi-arid/arid. Because of the high domestic demand for wheat, the drier areas are currently being developed for wheat cultivation. Preliminary experiments, followed by a few commercial pilot projects in farmers' fields, have shown that wheat can be produced in the drier regions of Kenya

with as low as 300 mm of rainfall per crop season (Anon, 1993). The existing Kenyan wheat cultivars, bred and selected under adequate rainfall conditions, may not necessarily be the most suitable for the dry areas. Thus, there is an urgent need to develop drought-tolerant wheat cultivars. The Government of Kenya through the Kenya Agricultural Research Institute approaches this problem by emphasising, as one of the viable strategies, the development of drought-tolerant wheat cultivars (Anon, 1991). To attain this goal, it is important to understand the techniques and yield-related traits that would be used for selecting drought-tolerant cultivars.

1.1.2 Adaptation of the Wheat Crop

Wheat is best adapted to moist and well-drained soils. In the temperate regions, wheat is grown where precipitation averages 1000 mm yr^{-1}. World-wide, about 75% of the land area under wheat receives an average of 625 mm of rainfall per annum. An annual rainfall of at least 700 mm (and no excessive evaporation) is considered ideal for growing wheat (Stoskopf, 1985).

The cultivation of wheat has spread to tropical and semi-tropical regions that have limited water supply. These regions differ in climate, soil type, agronomic practices, and environmental stresses associated with varying growing seasons (Stoskopf, 1985). Deviations from the optimal annual rainfall can cause abiotic stresses which can affect the phasic development of wheat (Begg and Turner, 1976). Further optimal rainfall values may not be adequate since high temperatures cause high transpiration rates. Many research reports (e.g., Nachit and Jarrah, 1986; Hadjichristodoulou, 1987) show that it is possible to modify crop plants to fit into drought environments. For many years, breeders and agronomists have accomplished this through improvements on phenological and morphological traits that can contribute to the adaptation of wheat in the tropics. Wheat can now be grown in drier areas where it was not expected to grow previously (Richards, 1991).

Wheat is classified to the genus *Triticum* and within this genus there are classifications made on the basis of chromosome sets. Wheat has three sets of chromosomes termed A, B and D. Some of the three classes of wheat are AA (*T. monococcum*, 2n = 2x-14), AABB (*T. turgidum* var. *durum*, 2n = 4x-28), and AABBDD (*T. aestivum* L., 2n = 6x-42) (Stoskopf, 1985). The two species that are of commercial value are: *T. aestivum* and *T. turgidum* var. *durum*. Most of the wheat grown throughout the world is hexaploid *T. aestivum* or, common wheat. The other wheat, *T. turgidum* var. *durum*, used for semolina products, is often called durum wheat. Generally, *T. durum* is regarded as more tolerant to drought than is *T. aestivum*. This adaptation would explain why over 75% of the total world durum wheat is grown in the Mediterranean regions that are characterised by severe drought (Annicchiarico and Mariani, 1996; Srivastava, 1984).

1.1.3 Breeding Wheat for Drought Tolerance

The development of drought-tolerant cultivars has been a major concern of crop scientists for many years (Blum, 1985; Clarke et al., 1994; Fischer and Maurer, 1978; Hurd, 1971). To make progress in this area is challenging because of the complexity of measuring and quantifying drought traits and other parameters associated with the traits themselves. According to Dedio (1975), developing drought-tolerant cultivars is slow because evaluations are done mostly in field trials where factors other than drought confound the problem. For example, thick epicuticular wax has been associated with increased grain yields and reduced rate of water loss through the cuticle (Johnson et al., 1983). However, this trait cannot be readily used to select for drought-tolerant cultivars because making accurate assays of epicuticular wax is so cumbersome. Besides, quantity may be less critical than the arrangement and chemical composition of the wax itself (Jordan et al., 1984).

The genetic variation in grain yield response to drought has been reported in many cases when measurements were conducted in terms of yield under drought (Bidinger et al., 1987; Fischer and Maurer, 1978). These studies used, amongst other criteria, the ratio of grain yield under drought to yield under non-stress conditions, the normalised stress/non-stress grain yield and the drought susceptibility index (DSI). The grain yield under drought conditions is influenced by environmental factors and the effects of genetic yield potential (Blum, 1988).

For the expression of traits related to drought tolerance, Blum (1985) outlined an approach designed to combine selection for yield potential in favorable conditions with selection under drought conditions. Subsequently, Balanos and Edmeades (1988) suggested that as appropriate criteria, whole plant or crop responses to drought (or "integrated traits") would be more effective than the "single trait" approach. However, the cost of using integrated traits, not to mention the difficulty of interpreting the results, probably makes this approach impractical.

Many wheat genes have been catalogued (McIntosh, 1988), but information on genes encoding for drought tolerance *per se* is lacking. For example, Dedio (1975), using controlled environment studies, reported that the heritability of water retention was under relatively simple genetic control. The excised leaf water retention trait seemed heritable and, under drought conditions, positively related to yield. A subsequent field study by Clarke (1986) showed that the parental lines used in Dedio's study did not differ significantly in water retention capacity. Such contrasting findings defeat any attempts to establish the genetic patterns of traits associated with drought tolerance.

Plant attributes associated with drought tolerance are elusive and it is difficult to use the classical genetics approach to resolve the genetics and inheritance of drought tolerance (Blum, 1988). The study of genetic control and inheritance of drought tolerance should therefore focus on the specific components of drought tolerance. According to Blum (1988), critical information in this respect is lacking. However, information on variation in specific drought responses is available for a limited number of traits, such as grain yield, osmoregulation, and proline content (Blum, 1988; Morgan, 1984). For example, Hanson et al. (1979) reported that it is possible to select for proline accumulation in the F_2 and F_3 generations derived from a cross between high proline and low proline barley genotypes. The high proline genotypes are characterised by a higher percent of leaf tip firing under drought conditions than are the low proline cultivars. However, there are still a number of issues, such as whether the accumulation of proline is constitutive or induced by drought. Although the whole process of proline accumulation is not fully understood, proline content may be inherited in a relatively simple way (Kueh et al., 1984).

Genetic variation for osmoregulation amongst wheat genotypes has been reported in many investigations (e.g., Blum et al., 1983; Morgan, 1991). Morgan (1984) proposed the existence of simple inheritance for osmoregulation and then suggested that wheat genotypes be classified into high, medium and low osmoregulators. These classes were derived from random F_4 progenies from a cross between high and low osmoregulating varieties. Blum (1988) noted that, although simple inheritance for osmoregulation has been proposed, it has yet to be established. After Morgan's (1991) studies, little research has been done in identifying the postulated gene(s) controlling osmoregulation. This raises questions concerning the nature of the solutes (such as sucrose, fructose, glucose, fructan and amino acids) involved in osmoregulation. How are these traits inherited? What genes control these traits?

Robertson et al. (1995) showed that *Hordeum* species when exposed to stress, produce proteins called dehydrins. The expression of these proteins is controlled by a dehydrin gene(s) in *Hordeum vulgare* L. cv. Himalaya. Despite the study done on dehydrin genes it is still not clearly understood how these genes are inherited, and there is little information on (1) how they interact with other genes, or (2) how the gene products function. Inadequate information on dehydrin genes has slowed down the use of these genes in practical breeding programmes, even though dehydrin genes have been identified in cereals.

1.1.4 Drought, Tolerance and Resistance

Ordinarily, any environmental factor capable of inducing a potentially injurious strain in plants may be defined as stress. A stress factor can be any external constraint that limits the rate

of dry matter production of all or part of the plant. Those factors that are of non-biological nature are grouped collectively as abiotic stress factors, whilst those of a biological nature are termed as biotic stress factors (Boyer, 1989; Johansen et al., 1994).

Plant growth under environmental stress requires physiological or biochemical adaptations. Such adaptations are expected to result in physiological, biochemical and morphological heterogeneity amongst plants. It has been proposed that the tolerance of a genotype may depend in part on its ability to divert respiratory resources from both growth and maintenance processes (Taylor, 1989). According to Taylor, stress-tolerant plants share a variety of characteristics, such as short stature and low inherent growth rates. However, this concept can not be generalised since not all short-statured plants are tolerant to stress, neither are all tall plants necessarily susceptible to stress.

For the growth and development of a plant, adequate water is requisite; a reduction of water content during the active life of plants affects physiological functions (Begg and Turner, 1976). In cereals and other crops such as legumes, approximately 25% of the water absorbed is used to maintain turgidity. Water makes up 85% to 90% of the fresh weight of the actively growing plant parts. Consequently, extreme dehydration can kill or adversely affect most plants (Leopold, 1990; Subbarao et al., 1995).

Water stress and drought. The two terms water stress and drought are often used interchangeably. The term drought has the advantage of clarity over the term water stress for drought can only refer to a deficit and never to an excess of water. Levitt (1980) defined drought as 'any combination of restricted water supply'. Drought can be caused by high atmospheric evaporative demand or as a result of low rainfall and poor soil water storage capacity.

Drought tolerance and drought resistance. The terms drought tolerance and drought resistance are often used interchangeably. Levitt (1980) defined drought tolerance as "the ability of plants to survive at low water potential". Fischer and Maurer (1978) defined drought tolerance as "all mechanisms that tend to maintain plant survival and productivity under drought conditions". Fukai and Cooper (1995) defined drought tolerance as a "mechanism by which some plants (tolerant) maintain metabolic processes even at lower leaf water potential, whilst other plants (susceptible) are severely affected". In an agricultural context, the more drought-tolerant a cultivar is, the higher its economic (e.g., grain) yield and the more stable its yield from season to season (Ludlow and Muchow, 1990).

1.1.5 Drought Effects and Symptoms

Drought symptoms can vary, depending on the stage of the crop, genotype involved, the intensity and duration of the drought. However, altered cell growth is the most sensitive response of the plant to drought, since cell growth is related to cell turgor. Cell turgor decreases with any dehydration-induced decline in cell water potential (Ball et al., 1994). During the early crop cycle of wheat, drought can cause plant stunting, decreased tillering capacity and root development. In the field, the leaves of plants under drought show curling or rolling, especially during mid-day. A reduction of spikelet and floret numbers might occur during spike development. Severe drought can cause poor grain setting and grain filling, and the result is reduced grain yield (Boyer, 1982).

1.1.6 Types of Drought

Varying types and levels of drought affect at least 25% of the 99 million ha of wheat cultivated in developing countries (Rajaram et al., 1996). There are many types of drought (Calhoun et al., 1994); thus, finding an appropriate environment for testing and selecting genotypes is difficult. Three different types of drought and regions have been identified (Kohli, 1985; Kohli and McMahon, 1988, Rajaram et al., 1996): (1) Early drought (ED) - The ED is characteristic of the southern cone of Latin America. This drought frequently occurs early in the growth cycle of wheat; moisture is often adequate or excessive during heading until maturity stages. (2) Late drought (LD) - The LD is characteristic of the Mediterranean region, where enough moisture is usually available during the initial growth stages. Plants therefore may be subjected to drought late in the growth cycle. (3) Residual moisture drought (RMD) - The RMD stress is typical of some parts of Australia and the India subcontinent, where wheat is normally produced using residual moisture (Rajaram, 1988; Woodruff, 1985).

Selecting genotypes for drought tolerance is a challenge because the rainfall patterns in drought regions is so erratic. In Kibwezi, Kenya, for example, the average annual rainfall is 645 mm, although during two peak periods rainfall may vary between 245 and 1200 mm. From March to April the average rainfall is 210 mm, and from November to December, 300 mm. During these two periods, the rainfall may be evenly or unevenly distributed (in the case of heavy storms). Neither of these peak periods is all that reliable. Nevertheless, two types of drought have been defined (pers. comm., KARI, unpublished data): (1) terminal drought (comparable to LD) usually occurs, with increasing intensity at the end of the growing season, or (2) intermittent drought which can occur at any time during the growing season. Because of these variations it is difficult to choose a particular stress protocol that would be best for the field selection of drought-tolerant genotypes in a breeding programme.

1.1.7 Quantification of Drought Tolerance

Research publications entirely concerned with the measurements of plant drought tolerance are exhaustive and have been summarised (e.g., Blum, 1988; Ludlow and Muchow, 1990; Subbrao et al., 1995). Many techniques have been considered for quantifying drought tolerance; several of these are discussed here.

Yield stability index. The yield stability index method was initiated by Yates and Cochran (1938), and was subsequently used by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). The method depends on linear regressions for determining cultivars that are adapted to targeted environments. This method involves testing cultivars across a wide range of environments and for many seasons to establish the yield stability coefficients of each cultivar. The method has drawbacks in that it is expensive and slow to produce results. Additionally, because of the high turn-over of test entries (spread over a number of seasons), interpreting the results can be challenging. However, yield stability analyses can represent the genetic variations in yield responses amongst genotypes and account for some of the interactions in both drought and non-drought conditions (Keim and Kronstad, 1979).

Superiority index. The superiority index (*P*) was proposed by Lin and Binns (1988). The *P* compares the productivity of genotypes across environments. This method uses the highest-yielding genotype within each environment as the standard. Thence, *P* instantly relates to the agronomic target of identifying genotypes with relatively high yield potential. Clarke et al. (1992), however, argue that until the *P* is evaluated more extensively, this approach is unreliable.

Genotypic desirability index. The genotypic desirability index, also known as area under the function, was first proposed by Hernandez et al. (1993). It combines the regression coefficient and the mean yield into a unified desirability index. The merits and drawbacks of this approach, which is as yet not widely used, remain unclear.

1.1.7.1 Drought Susceptibility Index (DSI)

The DSI developed by Fischer and Maurer (1978), and subsequently adopted for use in this study, can be summarised in the following equation [1.1]:

$$DSI = (1 - Ys/Yp) / (1 - Xms/Xmp) \qquad [1.1]$$

where DSI is the drought susceptibility index, Yp is the cultivar yield potential under non-drought conditions, Ys is the cultivar yield under drought conditions, Xmp is the mean of all test cultivars under non-drought, and Xms is the mean yield of all test cultivars under drought conditions. A major feature of the DSI is that it takes into account the yield under stress compared to yield potential of a given cultivar. The same researchers demonstrated that grain yield under drought

has a linear relationship with drought intensity. This finding prompted Fischer and Maurer to treat grain yield of each cultivar under drought as a function of yield potential (yield without drought), and intensity of drought. The cultivars showing lowest DSI are the most tolerant, whereas those with the highest DSI are considered the most susceptible to drought. Since Fischer and Maurer (1978) developed the DSI, other studies (e.g., Clarke et al., 1992; Hamdi and Erskine, 1996; Ndunguru et al., 1995) have used this index to describe drought tolerance in crops such as wheat and groundnuts.

1.1.7.2 Selection Methods and Drought Tolerance Traits

The literature is replete with field methods that have been used to screen wheat cultivars for drought tolerance. A brief summary of some of the common approaches used for identifying drought-tolerant cultivars is given here.

Selecting under natural environments. Selection for drought tolerance has been approached by selecting materials under natural environmental conditions (Davidson and Chevalier, 1990; Clarke et al., 1991; Van Oosterom and Acevedo, 1992). This approach involves testing genotypes over a number of generations to get representative dry or wet seasons upon which to base inferences. Large numbers of lines can be screened using this field strategy.

Staggering seeding date. In the field, selecting for drought tolerance can be approached by staggering the seeding dates (Bruckner and Frohberg, 1987; Clarke, 1992; Coventry et al., 1993), to force some part of the growing season into a period of high probability of drought. This method involves arranging whole experiments as separate units on the basis of planting dates. The assumption is that different seeding dates will be characterised with specific available precipitation or moisture. Thus, materials from different seeding dates with known precipitation or moisture levels can then be compared.

Selecting under harsh environments. Selection for drought tolerance under harsh environments has been reported (Ceccarelli et al., 1991). Genotypes that survive and produce the most grain are considered to be drought-tolerant. According to Ceccarelli et al. (1991) other morpho-phenological traits related to drought tolerance can also be identified under such harsh conditions.

Irrigated conditions. In a field environment, irrigation techniques can be used to evaluate wheat genotypes for drought tolerance (Clarke et al., 1992; Van Oosterom et al., 1996). Relative grain yields under rainfed conditions compared with irrigated conditions can be used to characterise cultivars or genotypes for drought tolerance. Another report (Rajaram et al., 1996) indicates that selecting for drought tolerance can be enhanced by selecting materials under both

dry and well-irrigated conditions. This approach is supposed to improve both yield potential and tolerance to drought.

Drought traits. A summary list of some of the traits that have been used to screen wheat cultivars for drought tolerance is presented in Table 1.1. In the present study, it is emphasised that, from a breeding standpoint, interest in physiological cause and effect (mechanisms) is of low priority as compared with which trait gives a significant correlation with drought tolerance.

Table 1.1. Summary of some traits (for wheat) reported to be associated with field drought tolerance studies.

Trait	Reference
Relative water loss	Clarke et al. (1991)
Root-shoot ratio	Hurd (1968, 1971), Richards (1991)
Biomass	Fischer and Maurer (1978), Sharma (1993)
Grain yield index	Clarke et al. (1992), Belay et al. (1993)
Epicuticular wax	Clarke and McCaig (1982)
Tillering capacity	Davidson and Chevalier (1990), Hucl and Baker (1991)
Plant height	Ehdaie and Waines (1996), Fischer and Maurer (1978), Richards (1991)
Seedling vigour	Acevedo et al. (1991), Fischer (1985), Souza and Sorrells (1991)
Ontogenic development	Garcia del Moral et al. (1991), Fischer (1985), Simane et al. (1993)
Days to heading	Acevedo et al. (1991), Van Oosterom et al. (1993)
Days to maturity	Fussell et al. (1991), Rasmusson (1991), Souza and Sorrells (1991)
Grain filling period	Acevedo et al. (1991), Simane et al. (1993), Souza and Sorrells (1991)
Kernels spike^{-1}	Chowdhry et al. (1996), Simane et al. (1993)
Kernels spikelet^{-1}	Acevedo et al. (1991), Rasmusson (1991), Simane et al. (1993)
1000-kernel weight	Fussell et al. (1991), Souza and Sorrells (1991)
Grain yield per unit area	Calhoun et al. (1994), Yau et al. (1991)
Days to anthesis	Fischer and Maurer (1978), Fussell et al. (1991)
Leaf rolling	Clarke (1986)

It is important to know the relevant plant traits that are associated with drought tolerance if one is to make meaningful selection (Blum and Ebercon, 1981). Such traits can only be of value to the breeder if they meet certain criteria (Ceccarelli et al., 1991): 1) there must be sufficient genetic variability, 2) they must have a high heritability, 3) they must have a good correlation with yield under drought, and 4) screening for them must be very easy and inexpensive. The number of traits individually proposed to be associated with drought tolerance is large (e.g., Turner, 1979; Ludlow and Muchow, 1990).

It is reported (Bidinger and Witcombe, 1989; Ludlow and Muchow, 1990) that all traits have not been critically evaluated and the necessary information to support the use of certain plant traits as selection criteria for drought tolerance is often inadequate. According to Ceccarelli

et al. (1991), improved grain yields under drought conditions can be realised by selecting cultivars using combinations of drought tolerance traits. For example, Van Oosterom and Acevedo (1992) reported that the use of individual traits as selection criteria in breeding programmes may be a futile exercise.

1.1.8 Leaf Relative Water Content

The relative water content (RWC) is the ratio of the amount of water in the leaf tissue at sampling to that present when the leaf is fully turgid (Smart and Bingham, 1974). This was originally described as 'relative turgidity' by Weatherley (1950) and can be measured accurately using the ratio of tissue fresh weight (FW) to tissue turgid weight (TW). It is widely accepted that RWC is a reproducible and meaningful index of plant water status (Clarke et al., 1991; McCaig and Romagosa, 1991). Further, Clarke and McCaig (1982) found that some drought-tolerant cultivars can retain more leaf water content than do drought-susceptible cultivars.

A subsequent study by Clarke and Townley-Smith (1986) showed that a high rate of water loss is negatively related to wheat grain yield under drought conditions. Clarke et al. (1989) showed that (1) low relative water loss from excised leaves was associated with higher grain yield of durum wheat under dry conditions, and (2) the rate of water loss can be used as a trait to classify cultivars into distinct drought-tolerant and -susceptible groups. Clarke et al. (1991) reported that the excised leaf water-loss rate is a moderately heritable trait and suggested that RWC can be used to some extent for the selection of drought-tolerant cultivars. In the current study, RWC has been used to measure the water status of the plant.

1.1.9 Electrolyte Leakage

According to Blum (1988), the effects of drought contribute significantly to metabolic and structural changes in plants. The structure and functions of the cell membrane are affected at the initial stages of drought. According to Steponkus (1984), the plasma membrane represents the ultimate barrier between the cytoplasm and the environment. It is the means by which metabolic control over all uptake processes is achieved. It is tempting, therefore, to speculate that variations between tolerant and susceptible cultivars may be related to differences in membrane characteristics. Membrane injury caused by stress has been documented (Steponkus, 1984), and according to Ferrari-Iliou et al. (1984) and Liljenberg (1992), stress disrupts the association between membrane lipids and proteins as well as enzyme activities. Once the membrane bi-layer integrity is affected, membrane permeability and micro-viscosity increase. This condition occurs in the tissue, when bout half the cells begin to plasmolyse, wherein the protoplast starts pulling away from the cell wall (Salisbury and Ross, 1991). Several cell structural changes are involved;

for example, the cell walls become folded. The plasma membrane usually maintains close contact with the wall even in the pleated state (Leopold, 1990).

According to Zhang et al. (1993), there are two stages that are involved in stress injury to the plant tissues; (i) functional injury leading to electrolyte leakage into the extracellular space, and (ii) structural damage leading to membrane disintegration. Sugars (e.g., glucose, sucrose, fructose and fructan), other solutes (e.g., potassium, calcium), and free amino acids (e.g., proline) leak out into the extracellular space after membrane disintegration (Evans et al., 1992; Martin et al., 1993; Santos-Diaz and Ochoa-Alejo, 1994).

When plant tissues are dehydrated, they show increased electrolyte leakage caused by damage to the cell membranes (Janowiak and Markowski, 1994; Srinivasan et al., 1996). High relative electrolyte leakage (EL) in stressed plant leaf tissue has been attributed to increased disorganisation and permeability of the cell membranes in response to water shortage (Quartacci and Navari-Izzo, 1992). It is of interest in this study to know whether electrolyte leakage could be used to differentiate tolerance of cultivars under drought conditions as well as to predict drought tolerance levels of different genotypes.

1.1.10 Chlorophyll

Chlorophyll content. The loss of photosynthetic pigments has been used to assess drought tolerance of cereal plants (Moustakas et al., 1993). A study by Quartacci and Navari-Izzo (1992) reported that Chl *a* content significantly decreased in drought-stressed cereal seedlings compared with the non-drought. The Chl *b* content did not change significantly under drought conditions. This observation was supported by Watanabe et al. (1994) who showed that wheat cultivars varied significantly in Chl *a* content under drought environments. However, the Watanabe et al. (1994) studies did not show any relationship between, for example, total chlorophyll (Chl T) and drought tolerance as assessed by DSI.

Chlorophyll fluorescence. The change of chlorophyll fluorescence has been reported as a trait that can be used to measure the level of plant stress (Bolhar-Nordenkampf et al., 1989; Schreiber et al., 1994). At normal temperature and without any plant physiological stress, the bulk of variable fluorescence (*Fv*) and maximum fluorescence (*Fm*) originate from photosystem II (PSII) which is mainly Chl *a*. Fluctuations in *Fv* and *Fm* reflect primarily the state of PSII under various environmental conditions. The *Fv/Fm* ratio is a measure of photosynthetic efficiency (PSE) (Bolhar-Nordenkampf et al., 1989). PSE changes in response to drought are reported in numerous studies (e.g., Dib et al., 1994; Van Rensburg and Kruger, 1993). Few of these studies have related PSE to grain yield and hardly any have related PSE to DSI. All this

research raises two questions: (1) What is the association between PSE and DSI? (2) Can PSE be used effectively as a screening trait for drought tolerance?

1.1.11 Osmoregulation in Plants

Osmoregulation is a specific form of solute accumulation that regulates turgor pressure and hydration during drought periods, with positive effects on growth (Hellebust, 1976). Solute accumulation in wheat is reported to be an adaptive strategy to drought conditions (Morgan, 1977). Other crop species such as barley (Blum, 1989), and sorghum (Santamaria et al., 1990) have also been shown to osmoregulate under drought conditions. In wheat, genotypic differences in osmoregulation tend to be discrete, with responses being either high or low (Morgan, 1984). Morgan (1983) selected wheat lines for higher osmoregulation in segregating populations in the greenhouse. The selected lines had greater growth and seed yields in water-limited conditions in the field. Morgan (1991) suggested that a single gene controls osmoregulation in wheat. Wheat genotypes with high osmoregulation capacity (e.g., cv. Chinese Spring) started to accumulate solutes immediately at the onset of drought, whereas the genotypes with low osmoregulation capacity (e.g., cv. Red Egyptian) did not start to accumulate solutes immediately at the onset of drought. Despite this successful study, the difficulties associated with osmoregulation studies and analyses have so far prevented the application of this trait in plant breeding .

Solute accumulation in fully expanded wheat leaves is reported (Jones et al., 1980) to contribute to the reduction of osmotic potential, resulting in partial turgor pressure. A summary of the major sugars and other solutes that account for osmotic potential is shown in Table 1.2. The osmotic potential contribution of total inorganic ions equals the total of sugars and amino acids. The studies of Jones et al. (1980) and Munns et al. (1979) show that solute accumulation is a complex trait and presents a challenge in discerning which solute to use for characterising drought tolerance in plant species at varying stress levels.

The major solutes that accumulate within the apex and expanding leaves of drought-stressed wheat are nonstructural carbohydrates and free amino acids (especially proline), which appear to be translocated from other parts of the plant (Munns et al., 1979). These compounds, that accumulate during the osmotic adjustment process, must be compatible osmotica as described by Yancey et al. (1982). Also, the osmotica should not affect the functioning of other plant components, such as enzymes, for metabolic processes of a plant (Cheeseman, 1988).

Table 1.2. Contribution of different solutes to the decrease in osmotic potential at full turgor relative water content = 100% and water potential ~ 0 MPa in sorghum leaves at two levels of stress.

	Mild stress	Severe stress
Pre-dawn water potential (MPa)	- 0.85	- 1.30
Reduction in osmotic potential at full turgor (MPa)	- 0.25	- 0.49
Osmotic potential accounted by increase in sugars:		
sucrose	- 0.04	- 0.05
glucose	- 0.04	- 0.06
fructose	- 0.02	- 0.03
Total free amino acids (TFAA)	- 0.02	- 0.04
Others (e.g., ions such as potassium and chloride)	- 0.13	- 0.23
Change in osmotic potential accounted for (%)	100	84

Data extracted from Jones et al. (1980). MPa = Megapascal.

1.1.12 Nonstructural Carbohydrates

Plants use non-structural carbohydrates (NSC), such as sucrose, fructose and glucose as sources of energy reserves. These reserves accumulate in different plant tissues, depending on the plant species and the environmental conditions. Non-structural carbohydrates are noted for the role they play in enhancing stress tolerance in many plant species (Levitt, 1980) and have been reported to increase in wheat cultivars grown under water deficit conditions (McCaig and Clarke, 1982; Virgona and Barlow, 1991). The NSC accumulate within the vegetative tissues of many plant species, such as wheat, in response to drought. NSC include: (i) ethanol-soluble carbohydrates, which are mostly mono- and di-saccharides, and low molecular weight oligosaccharides, and (ii) water-soluble carbohydrates, which are mainly fructan and high molecular weight oligosaccharides (Virgona and Barlow, 1991). For the purposes of the current study, the NSC examined are sucrose, fructose, glucose and fructan.

McCaig and Clarke (1982) found that the amount of NSC in wheat increased as the level of drought increased. The studies of Kulbauch and Thome (1989) and Pollock (1986) showed that the increase of NSC at various crop stages of wheat under drought conditions is mainly caused by the accumulation of fructan. Fructan is a polymer of fructose that is smaller in size than the polymers of glucose in starch. Structurally, fructan contain one terminal glucose unit which is synthesised by adding fructose units onto the fructose of a sucrose sugar molecule (Suzuki and

Pollock, 1986). Virgona and Barlow (1991) indicated that drought induced a decrease in the ratio of water-soluble to ethanol-soluble carbohydrates ratio, suggesting that fructan de-polymerisation is caused by drought-stress.

Approximately 15% of angiosperms produce and use fructan as the main storage carbohydrate. Amongst the angiosperms, some of the families that store and use fructan include the Poaceae (monocotyledons, e.g., barley and wheat) (Hendry, 1993; Nelson and Smith, 1986; Pollock and Cairns, 1991). Wheat and barley store fructan in the leaves and stems (Pontis and del Campillo, 1985, Van der Meer et al., 1994). Fructan is usually located in vacuoles and is partially or totally soluble in water (Brocklebank and Hendry, 1989). It has been proposed (Pollock and Cairns, 1991) that fructan in the vacuoles contributes to the osmotic potential of plant tissues. The presence of fructan can act as a protective mechanism against cold-induced desiccation and drought injury. Eventually, this can impart cold- and drought-stress tolerance in plants (Pilon-Smits et al., 1995; Van der Meer et al., 1994).

Munns et al. (1979) attributed the enhancement of osmotic adjustment to water-soluble carbohydrates (WSC). These accumulate in elongating parts of grass leaves responding to drought. Further, Volenec and Nelson (1984) reported that WSC make up approximately 50% of the total dry matter accumulated in regions of cell division and expansion in emerging leaf blades of grassy plants under water deficit. A subsequent study by Schnyder and Nelson (1987) attributed much of the WSC to fructan with a low degree of polymerisation (< 5). Fructan contributed approximately 0.2 MPa of the solute potential in actively elongating regions of drought-stressed plants. Questions of interest are whether differential genotypic changes of fructan levels under varying drought-stress level can be used to predict drought tolerance based on DSI. Can fructan and other non-fructan NSC, such as sucrose, glucose and fructose be effectively used directly or indirectly to select drought-tolerant cultivars? Is there any measurable relationship between NSC, as quantified during an early drought period, and drought tolerance measured in terms of grain yield?

1.1.13 Free Amino Acids

When plants are under drought stress, most amino acids occur in relatively smaller amounts than expected (Levitt, 1980). However, there are some amino-acid-related compounds (e.g., hydroxy-proline, and betaine) and amino acids (e.g., aspartic acid, asparagine, glutamine, phenylalanine, valine, alanine, and proline) that increase significantly when plants are under drought. Notably, free proline is usually high when grassy plant species, such as wheat, are under drought (Ashraf et al., 1991; Hunt, 1991).

Proline. In studies with excised perennial rye-grass leaves the free proline was observed to accumulate in wilted plant tissues (Kemble and MacPherson, 1954). Free proline has been accepted as one of the organic solutes that accumulates in a wide range of organisms, from bacteria to higher plants, when under stress conditions (Arora and Saradhi, 1995; Bassi and Sharma, 1993). The accumulation of proline occurs when plants are exposed to a wide range of environmental stresses, such as drought, or low and high temperatures. The accumulation of free proline is part of the process of osmotic adjustment that is important for cellular stress adaptation of many plant species such as wheat (Hanson and Hitz, 1982).

Proline offers protection as a non-toxic reserve of nitrogen and energy (Joyce et al., 1992). Palfi et al. (1973) reported that most plant species including wheat accumulate relatively high free proline under drought. The normal amount of free proline in plants is usually low, ranging between 0.2 and 0.7 mg g^{-1} dry matter. This rapidly rises to between 40 and 50 mg g^{-1} dry matter during slow dehydration of tissues. In wheat, an increase of proline and asparagine in the leaves has been associated with adaptation to severe conditions, such as winter stress or drought (Stewart and Bogges, 1978). Based on this information, proline was chosen as a study trait to measure its association to drought tolerance as defined by DSI. Questions that arise are (1) Can drought cause differential accumulation of free proline amongst wheat cultivars of diverse genetic background? (2) Can such free proline responses, if any, be used to predict drought tolerance based on DSI?

1.1.14 Total SDS-Soluble Proteins

Extreme drought can alter the capacity of plant tissues to carry out protein synthesis. This may indicate general cellular disturbance resulting from severe water loss (Bewley, 1981). Hsiao (1970) noted that a mild to moderate drought decreases the ability of plants to synthesise proteins, and that this capacity is regained on subsequent return to full hydration if drought is not too severe or long-term. Bewley (1981) showed that the more resistant a particular plant or tissue is to drought, the longer it can continue to synthesise proteins and maintain normal biological functions. Some reports (e.g., He et al., 1995; Pino et al., 1995; Robertson et al., 1995) have shown that drought causes a significant increase of protein in barley, maize and wheat plants. However, these reports do not show how the expressed proteins correlate, for example, with wheat grain yield under drought, or with DSI. This leads to the question whether differential total SDS-soluble protein responses to drought can be found amongst genetically diverse wheat cultivars and, if so, would such differences be useful for predicting drought tolerance based on DSI?

1.1.15 Nitrate Reductase (EC 1.6.6.2)

Drought is one of the factors that affects the conversion of nitrates to nitrites in plants, causing an accumulation of nitrates in plant tissues (Hanway and Englehorn, 1958). Reports (Mattas and Pauli, 1965; Mayo 1895) that nitrate accumulation in plants grown under drought conditions was responsible for livestock poisonings led to the early studies on the effects of drought on nitrate reductase (NR).

Nitrogen enters the plant metabolism from the soil in the form of nitrate (oxidation state +5) and finds its way first to glutamic acid and via transamination to other amino acids. From these the majority of other nitrogeneous compounds are synthesised. Thus, nitrate has to be reduced by plants to nitrite (oxidation state +3), using NADH or NADPH as reducing substrate, according to the following equation [1.2]:

$$NO_3^- + NAD(P)H + H^+ \rightarrow NO_2^- + NAD(P)^+ + H_2O \quad [1.2]$$

The first reaction step from nitrate to nitrite is catalysed by NR, which is found in plant leaves and other tissues (Kaiser and Huber, 1994; Sanderson and Cocking, 1964). The enzyme NR is located at the crossroads of two energy-consuming pathways: nitrate assimilation and carbon fixation. Hageman et al. (1967) identified NR as a rate-limiting step in nitrate assimilation. Beevers and Hageman (1969) further suggested that NR was the point where the regulation of the input of reduced nitrogen in higher plants including wheat takes place because it is (i) the first enzyme in the nitrate reduction pathway, (ii) the rate-limiting step of nitrate reduction, and (iii) substrate-inducible. According to Hageman et al. (1967) there are differences in nitrate reductase activity (NRA) amongst crop cultivars of e.g., maize and wheat, resulting in the ability of some cultivars to accumulate more nitrates than others. According to Hageman et al.(1967) the inhibition of the growth of cells, leaves and whole plants under drought is partly associated with the accumulation of nitrate in the plant tissues, particularly in leaves. Balusubramanian et al. (1974) reported that NR in wheat and barley has a longer half-life than in other plant species. In the exclusion of both light and nitrate, the half-life of wheat NR can range between 33 h and 67 h at 20 °C. It is of interest to know whether NRA can be used for predicting drought tolerance amongst wheat cultivars that are droughted under controlled conditions. Other questions of interest are: (1) Can drought cause differential responses in NRA amongst wheat cultivars? (2) Can NRA be used to predict drought tolerance in wheat given the importance of this enzyme in influencing a wide range of metabolites?

1.1.16 Grain Yield and Grain Yield Components

Grain yield. Grain yield can be analysed in terms of three yield components (1) the number of spikes per unit area, (2) the number of kernels per spike, and (3) mean kernel weight. These components develop chronologically, with later components being determined by preceding ones (Dofing and Knight, 1992) and interacting in compensatory patterns, especially under drought conditions (Garcia del Moral et al., 1991).

Grain yield is a complex trait that represents the total expression of the entire genome of a cultivar and its interaction with the environment (Blum, 1988; Hadjichristodouluo, 1986). To gain some knowledge about grain yield, especially for cultivars under drought conditions, it is necessary to understand the contribution of individual grain yield components to grain yield. Difficulty in achieving this arises because the determinants of the yield components, and physiological understanding of the nature of the interaction between the yield components, are not in place. Further complications arise because naturally occurring drought environments are variable and not repeatable. To obtain accurate results requires the testing of many cultivars across a wide range of locations for many seasons. A multi-year, multi-site testing approach introduces a genotype x environment component, thus complicating the interpretation of grain yield results.

Under drought, grain yield is dependent upon many phenological, morphological, and physiological characters (Ludlow and Muchow, 1990) that can collectively contribute to drought tolerance. This is why in the absence of an understanding of specific mechanisms and components of drought tolerance, quantification of drought tolerance should be based on grain yield under dry conditions, according to Fischer and Maurer (1978).

Bidinger et al. (1987) argued that grain yield obtained following a drought period is not an accurate measure of drought tolerance. This is because genotype differences in grain yield potential and drought escape both have large influences on yields. Some studies (Fussell et al., 1991; Rajaram et al., 1996) have suggested that in order to improve grain yield for drought environments, selection should be done under optimal conditions. On the other hand, Rajaram et al. (1996) argue that wheat improvement should be done by combining factors for high grain yield potential and adaptation to drought in one genotype. This is contrary to Ceccarelli et al. (1987) who advocate that wheat materials targeted for drought environments should be screened specifically under drought conditions.

Biomass. There are numerous studies (e.g., Fischer, 1985; Shepherd et al., 1987) on small grain cereals (such as wheat) that have shown that genotypes having relatively high biomass

at pre-anthesis and anthesis yield more grain than genotypes with low biomass. According to Acevedo et al. (1991), many of the traits associated with grain yield under drought conditions are fixed during early crop development stages. In another study, Hadjichristodoulou (1986) proposed that the ideal cultivar under drought conditions is one that would allocate the highest proportion of assimilate to the production of grain. The questions that arise are: (1) Can biomass amongst a wide range of wheat cultivars show differential response to drought conditions? (2) Can such responses be used to predict drought tolerance based on DSI?

Thousand-kernel weight (TKW). Before a review of TKW is attempted it is important to note that in cereal crops (such as wheat) the actual kernel weight is not the same as the potential kernel weight. This is because of the competition for the available assimilates and interplant competition for resources such as water (Bruckner and Frohberg, 1987). According to Briggs (1990) and Bruckner and Frohberg (1987), kernel weight in cereal spikes generally increases in response to reduced kernel number per spike. Furthermore, Briggs (1990) showed that kernel size was significantly influenced by the grain-yielding capacity of a given cultivar. It is assumed that the kernel weight increases or decreases because of the availability of assimilates to each kernel and this depends on the environmental conditions where the cultivar is growing. For example, drought reduces the availability of assimilates to kernel filling, thus resulting in kernels of relatively low weight (Fischer and HilleRisLambers, 1978). This would eventually affect TKW. According to Bouzerzour and Benmahamad (1991), high TKW is associated with high grain yield under drought conditions. As yet, however, TKW has not been tested as a predictor of DSI on a wide range of wheat cultivars from varying genetic backgrounds. However, there is little prior evidence to suggest that drought tolerance assessed by DSI is related to TKW, or TKW response to drought.

Kernels spike^{-1}. The number of kernels spike^{-1} is a reflection of the differentiation and mortality of spikelets and florets during the early stages of plant development. The number of kernels spike^{-1} also depends from the tiller from which the spike was obtained, the genotype, and environmental conditions (Briggs, 1990; Hay and Kirby, 1991). For example, under drought, florets in the spikelets at the extremities of the spike may show retarded development, and die at a relatively early stage, eventually resulting in reduced number of kernels spike^{-1} (Langer and Hanif, 1973). It is, therefore, of interest to know whether wheat cultivars can be differentiated by using the response of the number of kernels spike^{-1} to drought and, if so, whether such responses can be used to predict drought tolerance.

Tillering. The number of fertile tillers per plant is a primary determinant of grain yield in wheat. Davidson and Chevalier (1990) determined that water deficit can cause wheat grain yield reductions partly because of the accompanying reduction in the number of tillers that bear grain (i.e., become the fertile spikes). It is possible that even under adequate moisture conditions many tillers may not survive to maturity to produce grain. The loss of potential kernel-bearing tillers can reduce the sink capacity and this can affect the grain yield of wheat cultivars. Kulshrestha and Chowdhury (1987) reported that dry matter (including grain) losses of up to 36.5% can be attributed to tiller mortality in spring wheat cultivars that were grown under different management regimes, such as water deficit conditions. Theoretically, under water deficit conditions, plants with fewer tillers may save water as a result of reduced canopy development before anthesis, and thus improve their moisture supply during the grain filling period. This theory may not hold true, as other factors such as the change of leaf development can increase as tiller numbers decrease. Hucl and Baker (1991) found that under drought conditions common to the semiarid regions of Western Canada, oligoculm spring wheat plant types did not grow and produce grain any better than normal tillering cultivars. This was partly because the tiller mortality of oligoculms was higher than that of the free tillering cultivars. According to Van Sanford and Utomo (1995), the development of low-tillering genotypes can be useful in improving yield under water deficit conditions.

Bunting et al. (1964) described tillering as a "plasticity in the plant." Tillering allows the plant to adapt to various environments from season to season. Thorne (1966) found that even where the climate is not arid, the production of fewer tillers was associated with better-yielding cultivars. Thus, a breeding programme for drought environments should target plants that have fewer tillers and that mature at about the same time. It is not, however, easy to breed cultivars that have specific numbers of tillers for specific drought environments. Furthermore, it is impossible to estimate confidently how many tillers constitute the "optimal" number in a particular environment. According to Van Sanford and Utomo (1995), the heritability (h^2) of tiller numbers in a single environment has been reported to range from 0.05 to 0.60. When genotype x environment interaction was accounted for in a combined analysis across environments, heritability estimates were much lower, ranging from - 0.14 to 0.19. The same study showed that under drought conditions, grain yield still could be predicted (r = 0.58*) indirectly by selecting for tiller numbers despite the low h^2 associated with tillering. Questions that remain are: (1) Do wheat cultivars show significant differential responses for tiller number under drought

conditions? and (2) Can such responses be used to predict drought tolerance as quantified by DSI?

Plant height. There are many reports (e.g., Ehdaie and Waines, 1996; Law, 1995) that have demonstrated that the height of wheat is an important trait to consider, especially when screening cultivars under drought conditions. The short-statured cultivars popularly known as the semidwarfs are closely associated with the Green Revolution (Borlaug, 1968; Law, 1995). According to Law (1995), the semidwarfs have genes, such as *Rht1* and *Rht2*, that are responsible for the improved wheat grain production, especially in developing countries where drought is one of the major limiting factors. Furthermore, Law (1995) demonstrated that the degree of reduction of height is correlated with the numbers of kernels set within the spike. The shorter the plant the higher the number of kernels spike^{-1}. However, this does not mean that the shortest wheat cultivars will be the highest grain producers as cultivars must also have the grain filling capacity.

For this trait (plant height), questions that remain for breeders include: (1) Do cultivars show plant height differential responses to drought? and (2) Can such height responses be used to predict drought tolerance based on DSI?

1.1.17 This Study

It was known from the onset of this project that there are various types of responses that can determine wheat cultivar adaptation to drought. The responses are: (1) drought escape, (2) drought avoidance, and (3) drought tolerance (Ludlow and Muchow, 1990). Rapid development during periods of high water availability can allow cultivars to escape drought periods. Drought-stress avoidance mechanism can be obtained by a reduction of water loss. This can be achieved through lowering of stomatal conductance, and continuation of water uptake by increased root density and depth. Drought tolerance allows plants to maintain normal physiological functions despite a low water potential in the plant, and may involve mechanisms such as turgor maintenance by osmoregulation (Morgan, 1984).

I chose to study drought tolerance for various reasons. Studying drought tolerance is relatively less cumbersome than studying drought avoidance or escape where one might have to, for example, assay roots or measure stomatal conductance. This may not be practical in a wheat breeding programme where handling large numbers of samples is the norm. Also, I chose to study drought tolerance because there is prior literature showing reliable indices such as the DSI (Fischer and Maurer, 1978) for quantifying drought tolerance. In contrast, published literature is litle showing drought avoidance or drought escape indices.

In the present study, the focus on the effects of drought on eight wheat cultivars of diverse genetic background that were selected on the basis of putative differential drought tolerance. These cultivars were studied under two water treatments: (1) non-drought, and (2) drought in three replicates. The non-drought was maintained at field capacity (i.e., 0.018 kg of water kg^{-1} dry wt. growing medium). The drought treatment was imposed in two cycles by withholding water. The first drought was initiated 16 days after planting (DAP) and lasted four days. This was an acclimating treatment. The second drought was imposed at 20 DAP by withholding water until 30 DAP and maintaining a 0.06 kg of water kg^{-1} dry wt. growing medium.

The imposed drought was analogous to ED (early drought) described in Section 1.1.6. Electrolyte leakage (EL), Chl *a*, Chl *b*, Chl T, Chl F, sucrose, glucose, fructose and fructan, total SDS-soluble proteins (TSP), TFAA, free proline and nitrate reductase were assayed at 30 DAP. At plant physiological maturity, the effects of ED stress on yield-related traits, such as grain yield, biomass and grain yield components were evaluated. The grain yield components included the thousand-kernel weight (TKW), the number of kernels $spike^{-1}$, the number of fertile spikes and the number of kernels $plant^{-1}$. Other yield-related traits, such as total number of tillers and plant height were also measured. The yield-related traits that were assayed at 30 DAP and at maturity were tested to determine whether they would predict drought tolerance, where drought tolerance was defined on the basis of drought susceptibility index (DSI) as described in Section 1.1.17.

The overall approach was to measure selected variables previously described in the literature, in the same experiment under the same drought conditions, to determine the relationships with the yield-based drought tolerance trait DSI, which was determined in a separate experiment under the same drought protocol.

1.1.18 The Study Framework

The framework of the present study was founded on the fact that in prior published literature drought studies had been focussed on individual traits and had not evaluated the relative utility of any given trait as a predictor for drought tolerance quantified by DSI (e.g., He et al., 1995; Palfi et al. 1973, Virgona and Barlow, 1991). A different approach of conducting biological assays of eight wheat cultivars at similar tiller (main shoot and six tillers) growth stage was used. This growth stage was equivalent to Haun Scale 7.+ for all cultivars (Haun, 1973). This growth stage was selected purposely because it is when yield components such as tillers and spikes are increasing in a linear manner, and is a period when wheat is very susceptible to drought

(Wardlaw, 1967). All parameters measured were then related to DSI, as a measure of drought tolerance.

To assess the effects of drought on wheat cultivars and make meaningful selection for drought-tolerant cultivars, it is important to understand which plant traits are affected by drought. It is also important that all other factors that may confound the response of such traits be identified and separated to attain meaningful results (Blum, 1988). Thus, conducting studies under standardised stress non-drought conditions is necessary in identifying drought-tolerant genotypes. Also, I wanted to learn about the possibility of increasing predictability of drought tolerance by taking advantage of any identifiable additivity of individual traits. This could only be studied by examining all traits at the same time, under the same drought protocol.

The Study Hypothesis. The hypothesis of the current study was that biological assays conducted 30 days after planting (DAP) on wheat cultivars exposed to early drought would show response to drought and could then be used to predict drought tolerance measured as drought susceptibility index (DSI). This hypothesis was extended to grain yield traits, including number of tillers, and plant height at maturity. One or more of these traits was expected to respond to drought and therefore to be useful in predicting DSI, and existence of some additivity of traits for prediction purposes was hypothesised.

Objectives. The objectives of this study were: (**1**) to assess the effects of drought on eight wheat cultivars of diverse genetic background and reported drought tolerance level. This required measuring the responses of the traits to altered drought conditions, using many parameters, including relative water content (RWC), electrolyte leakage (EL), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl T), photosynthetic efficiency (PSE), sucrose, glucose, fructose, fructan, total free amino acids (TFAA), free proline, nitrate reductase activity, total SDS-soluble proteins (TSP), grain yield and grain yield components, and DSI under controlled environment, and (**2**) to determine the relationships between the listed traits and assess whether these traits can be used effectively for selecting or predicting drought-tolerant wheat cultivars based on DSI.

The fundamental questions posed in this study were: (1) Can drought at the tillering stage cause differential effects on the eight selected wheat cultivars? (2) How will the eight wheat cultivars respond when exposed to drought? (3) If various assays are conducted simultaneously at 30 DAP after an early drought, can combinations of such assays better predict drought tolerance based on DSI? (4) What relationship exists amongst the assays conducted simultaneously on the wheat cultivars after an early drought? and (5) How will drought at tillering stage affect grain

yield and grain yield components and which grain yield components could be used to predict DSI?

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CHAPTER 2

DROUGHT-INDUCED CHANGES IN CARBOHYDRATES, AMINO ACIDS, PROTEINS AND NITRATE REDUCTASE ACTIVITY IN WHEAT CULTIVARS

2.1 INTRODUCTION

Drought is a major constraint to wheat production worldwide. Studies have shown that wheat yield is differentially reduced by drought. This depends on the drought tolerance level of a cultivar and drought intensity, the plant growth stage, and environmental factors including temperature (Hucl and Baker, 1991; Moustafa et al., 1996; Yau et al., 1991). Drought-tolerant cultivars can be described as those with relatively low drought susceptibility indices (DSI) as described by Fischer and Maurer (1978). DSI estimates relative stress injury accounting for variation in yield potential and stress intensity (Bruckner and Frohberg, 1987).

To ensure that stress was present and that there was no drought escape or avoidance, relative water content (RWC) was used as a measure of drought. This is an acceptable, repeatable and meaningful index for determining the plant leaf water status (McCaig and Romagosa, 1991).

Studies have shown that drought influences many plant metabolic processes. Under drought, barley and wheat accumulate proteins (Robertson et al., 1995). Under field drought conditions, using two wheat and two oat cultivars, McCaig and Clarke (1982) demonstrated that non-structural carbohydrates increased or decreased, depending on the cultivar and plant growth stage. Field studies on barley, wheat and oats have shown that drought can increase or decrease leaf tissue fructan, sucrose, fructose and glucose depending on the species, growth stage and intensity of the drought itself (Olien and Clarke, 1993).

Drought also leads to the accumulation of amino acids, especially proline (Hanson and Hitz, 1982). Morgan (1978), using six wheat genotypes, demonstrated that there were genotypic differences for the capacity to accumulate amino acids. However, these studies did not investigate whether proline was correlated with DSI. Dingkuhn et al. (1991), using seven rice cultivars, demonstrated that proline accumulation was an osmoregulation strategy of plants to cope with stress.

Nitrate reductase is the first enzyme in nitrate assimilation, a critical process for plant survival. The activity of nitrate reductase (NRA) in higher plants, including wheat, is sensitive to drought (Kaiser and Huber, 1994). Also, wheat cultivar differences in NRA exist and vary depending on the plant growth stage (Hageman et al., 1967; Sinha and Nicholas, 1981). Feil et al. (1993) have demonstrated genetic variations in NRA using maize cultivars of diverse genetic

backgrounds. They showed that environmental conditions such as drought and temperature were the main determinants of NRA.

Wheat breeders would like to develop drought-tolerant cultivars. To achieve this, breeders have attempted to select for physiological traits that would be associated with optimal plant-water relations of wheat under drought conditions.

Studies that examine multiple trait predictors assayed in a range of cultivars grown under common drought conditions are rare (e.g., Dib et al., 1994). Further to this, potential interactions between predictors have not been investigated as each predictor reflects different potential modes of tolerance. The Dib et al. (1994) study, using 25 durum genotypes from the Mediterranean dry lands, showed that DSI was significantly and negatively correlated with proline, grain yield, biological yield, TKW and tiller numbers. For purposes of this study, several methods of assaying drought tolerance were used on a common set of wheat cultivars to determine if they can predict drought tolerance level and possible relationships between them.

Hypothesis. The hypothesis of the study was that there would be significant association between DSI and sucrose, glucose, fructose, fructan, free proline, total SDS-soluble proteins (TSP), total free amino acids (TFAA) or nitrate reductase activity (NRA).

Objectives. The objectives of the present study were: (1) to investigate whether there were differential responses to drought for sucrose, glucose, fructose, fructan, free proline, total SDS-soluble proteins (TSP), total free amino acids (TFAA) and nitrate reductase activity (NRA) amongst eight wheat cultivars of known diverse genetic background, when evaluated under a common drought protocol, and (2) to evaluate whether the listed variables in (1) can be used to predict drought tolerance based on DSI.

2.2 MATERIALS AND METHODS

2.2.1 Drought susceptibility index (DSI)

DSI (Fischer and Maurer, 1978) was calculated for each cultivar using the equation described in Chapter 1, Section 1.1.7.1. A separate experiment using a common drought protocol was used to derive DSI values. This was performed to avoid autocorrelation of data. After 30 DAP, the drought-stressed plants were watered similar to the non-drought. In the non-drought treatment, available water was maintained at 0.18 kg kg^{-1} dry wt. growing medium throughout the experiment, until physiological maturity. At physiological maturity, grain yield was measured and used for computing DSI as described by Fischer and Maurer (1978).

2.2.2 Cultivars

Some details about the experimental spring wheat cultivars are provided in Table 2.0. Two durum wheat cultivars (*T. turgidum* var. *durum*, cv. Pelissier, and Hercules) and six common bread wheat cultivars (*T. aestivum* L., cv. Pitic 62, Biggar, K. (Kenya) Mbweha, K. Nyangumi, Pasa, and Kwale) were evaluated. The cultivars were selected primarily to represent a perceived range of drought tolerance, considered on the basis of prior literature, genetic diversity, selection history, pedigree and morphological differences.

Table 2.0. Spring wheat cultivars tested.

†Cultivar	Parentage/Pedigree	Origin	‡Drought characteristic
1. Pelissier	A land race introduced via USA and released in Canada	Algeria (1929)§	(T), Low residual transpiration rate (Clarke and Richards, 1988)
2. Hercules	RL3097-RL3304/SR-LD393	Canada (1969)	(S), Low water retention (Clarke et al., 1991)
3. Biggar	Tobari 66/Romany = HY320 Sel.	Canada (1989)	(N), Stable yield under variable conditions
4. Pitic 62	Yaktana54/Norin10/Brevor126-C	Mexico (1962)	(T), High grain yield under drought (Hurd, 1974)
5. K. Mbweha	CI8154/2*Fr/3/*Gb5	Kenya (1973)	(N)
6. Pasa	Buc"s"/Chat"s"	CIMMYT (1989)	(Syd)
7. Kwale	Kinglet"s" = CM33089	CIMMYT (1987)	(N)
8. K. Nyangumi	Tzpp//Ske/Lr64Hdm/3/Afm/4/Ksw/K45006	Kenya (1979)	(N) Possibly tolerant?

†1-2 = Durum (tetraploids), and 3-8 = bread wheat (hexaploids) cultivars. All cultivars are spring-type
‡Literature-based putative drought tolerance level. (T) = Tolerant, (S) = Susceptible.
(Syd) = Severe yield decrease under dry land wheat production in Kenya (pers. comm.).
(N) = No published information known. §Numbers in parenthesis indicate year of release.

Tetraploids. Pelissier has been reported to be drought-tolerant because of its low residual transpiration rate or high water retention capacity (Clarke and Richards, 1988), effective leaf rolling resulting in reduced surface area for transpiration (Clarke, 1986) and an extensive root system development under drought conditions (Hurd, 1971). Hercules is known to be drought-susceptible based on the lesser expression of the leaf rolling characteristic under drought (Clarke, 1986). Hercules has a high residual transpiration rate (Clarke and Richards, 1988) and low grain yield under drought conditions (Clarke et al., 1991). However, there is lack of information on

how these cultivars respond to drought with glucose, fructose, fructan, TFAA, free proline, TSP and NRA.

Hexaploids. Pitic 62 is classified as drought-tolerant, based on high grain yield and low residual transpiration under drought-stress conditions (Clarke and Richards, 1988; Hurd, 1971, 1974). Biggar represents the Canadian Prairie Spring (CPS) class, a widely grown semidwarf variety with stable yield performance (pers. comm., Briggs). Kwale and Pasa represent popular wheat cultivars in Kenya that contrast in yield potential under dry-land wheat cultivation. Pasa has low grain yield compared to Kwale under Kenyan dry-land wheat cultivation (unpublished data, Kenya Agricultural Research Institute, Kenya). Thus, Pasa represents the drought-susceptible hexaploid wheat (pers. comm., KARI). K. Mbweha and K. Nyangumi represent the typical genetic background of most wheat cultivars bred and selected under generally drought-free Kenyan breeding programme conditions, but for which no prior description of drought response is documented.

2.2.3 Plant Growth Conditions

Wheat seeds were surface-sterilised using 1.2% sodium hypochlorite for 5 minutes. Within each cultivar, seeds of similar size were germinated for 2 days in the dark at 28 °C. The seeds were covered in petri dishes on water-soaked filter paper. Four germinating seeds were selected and planted in 210 × 210 mm (diameter/depth) plastic pots that had four drain holes at the bottom. Each pot was filled with 1.5 kg of growing medium consisting of a mixture of peat-moss, coarse vermiculite, coarse sand in a ratio of 1:1:1 by volume. The growing medium was supplemented with a basal fertiliser containing: 1:1 mixture of: $CaCO_3/MgCO_3$, 10.8 g kg^{-1}; super phosphate (0-20-0, $Ca(H_2PO4)_2$), 5.6 g kg^{-1}; Nutricote® (14:14:14, $N:P_2O_5:K_2O$), 9.6 g kg^{-1}; iron chelates, 0.08 g kg^{-1}; micronutrients (B, 0.39; Cu, 0.03; Fe, 2.1; Mn, 0.6; Mo, 0.018; Zn, 0.12 mg kg^{-1}) (Plant Products Co. Ltd., Brampton, ON).

The plants were grown in a growth room at 21 °C (light) and 19 °C (dark) with 16-h photoperiod. Illumination consisted of a blend of fluorescent (Sylvania, 115W, Cool White), and incandescent (100W) lamps. Plants received photosynthetic active radiation of 1350 µmol m^{-2} s^{-1} at the top of the leaf canopy and relative humidity was maintained at 65 ± 6.9%. The plants were fertilised once fortnightly with a complete commercial fertiliser (20-20-20, $N:P_2O_5:K_2O$) (Plant Products Co. Ltd., Brampton, ON) at the rate of 0.2 g pot^{-1}. To correct for micro-nutrient deficiencies, a commercial fertiliser solution containing micro-nutrients (B, Cu, Fe, Mn, Mo, Zn) (Plant Products Co. Ltd., Brampton, ON) was sprayed to lightly cover the leaf surface. The

manual watering was maintained to keep pots at field capacity until 16 days after planting (DAP) when drought treatments were initiated.

Drought treatments. Early drought was induced at 16 DAP by withholding watering for four days. The plants were watered again to field capacity at 20 DAP, after which they were then subjected to a second period of drought, leading to severe symptoms of wilting at 30 DAP when various assays were conducted. Throughout the experiment, the non-drought plants were watered daily to field capacity. The field capacity water requirements were determined by standard gravimetric assay procedures. The non-drought treatment field capacity of the growing medium used in this study was maintained at a water concentration of 0.18 kg kg^{-1} dry wt. growing media. The drought treatment was maintained at a water concentration of 0.06 kg kg^{-1} dry wt. growing medium. Watering involved weighing each pot and determining how much water was needed to bring pots to the required field capacity level.

2.2.4 Leaf Relative Water Content

The water content in the growing medium *per se* and leaf relative water content (RWC) were used to establish a basis for evaluating plant drought tolerance. Using leaf RWC during harvest and assay time, drought level was estimated for both the non-drought and drought-stressed plants. The youngest fully emerged and expanded leaves (YFEEL) from the main stems were harvested at 0900 h 30 DAP, and RWC was estimated on four leaf blades weighed immediately after harvest to determine their fresh weight (FW). The leaf blades were placed in test tubes containing double-distilled water and left to stand on a laboratory bench at room temperature for 4 h before turgid weight (TW) was recorded. The leaf blades were placed in marked envelopes and oven-dried at 75 °C for 24 h after which dry weight (DW) was measured. RWC was calculated using the method described by Smart and Bingham (1974) equation [2.1]:

$$\%RWC = [(FW - DW)/(TW - DW)] \times 100 \qquad [2.1]$$

where FW is fresh weight, DW is dry weight, and TW is turgid weight. The RWC of cultivars in the non-drought treatment was 95.9 ± 3.7 (mean ± SE, n = 24), whilst the RWC of the cultivars in the drought treatment was 73.1 ± 3.6 (mean ± SE, n = 24).

2.2.5 Carbohydrates Quantification

The nonstructural carbohydrates were extracted, using the protocol developed by Hendrix (1993) with some modifications. The freeze-dried leaf tissue was ground in liquid nitrogen to a fine powder using a pre-cooled mortar and pestle. Approximately 200 mg of ground leaf tissue was placed in 10-mL culture tubes. The samples were incubated in 5 mL of 80% ethanol for 20 min at 75 °C to inactivate the enzyme invertase. The samples were constantly agitated during the

incubation period. After the incubation period, the contents were centrifuged for 5 min at 1,000 x *g* and the supernatant was removed. This procedure was repeated five times and all extracts were combined. The pooled contents were concentrated under reduced pressure at a constant temperature of 38 °C using a rotary evaporator. The carbohydrate extracts were redissolved in 1.5 mL of deionised double-distilled water and kept in the refrigerator at 2 °C. All the samples were analysed by HPLC within five days of extraction.

The carbohydrates were quantified by the HPLC method described previously by Karsten et al. (1991), with some modification. The concentrated (1.5 mL) samples were run through sequential ion exchange columns. These columns contained Bio-Rad® AG-50W-X8 H^+ (strong acid cation exchange resin) and AG-2-X8 Cl^- (strong anion exchange resin) to remove any ionic contaminants from the extracted samples. Ionic contaminants, if not removed, can damage the analytical column and interfere with the separation and quantification of carbohydrates. The neutral carbohydrates were washed through the resins using 15 mL of deionised water and then dried under reduced pressure at 38 °C. The carbohydrates were redissolved in 1 mL of HPLC-grade water and filtered through a Nalgene® syringe with < 0.45 mm pore size nylon filter.

The non-structural carbohydrates constituted sucrose, glucose, fructose and fructan. These were separated and quantified by HPLC. The HPLC consisted of a Shimadzu® - LC 6A coupled with a SLC - 6B controller unit. The separation of the sugars was achieved using a Rezex® RCM-monosaccharide column (Phenomenex®, Cat. No. 00H-0130-KO, Torrance, CA). The stainless steel column (300 mm x 7.8 mm) was packed with 8% cross-linked resin in the calcium ionic form and had a pore size of 10 µm. The column was operated at a constant temperature of 75 °C using a column block heater (Eppendorf® CH-430). A 50 mm x 7.8 mm Phenomenex® guard column (Cat. No. 03B-0130-K0) containing similar packing material as the analytical column was connected to the analytical column. The guard column was kept at room temperature (about 21 °C). Degassed HPLC-grade water maintained at a constant flow rate of 0.6 mL min^{-1} was used as the mobile solvent phase. Samples were injected into the HPLC using a 10-µL Rheodyne® loop. The sugars were detected with a Shimadzu® RID-6A differential refractometer that was maintained at a constant internal temperature of 35 °C. The sample carbohydrates were quantified using external standards of sucrose, glucose, and fructose. Inulin was used as the standard for fructan. Peak areas were determined using a Shimadzu® C-R3A chromatopac integrator. Retention times in minutes for each sugar were as follows: fructan (inulin) fraction, 8.9; sucrose, 11.5 (MW = 342.2); glucose, 13.6 (MW = 180.2); and fructose, 18.2 (MW = 180.2).

The extraction efficiency was checked by spiking several trial samples with 20 µg α-L-rhamnose (6-deoxy-L-mannose, MW = 164.2, Sigma®, product No. R-3875). Rhamnose, a sugar molecule not found in wheat leaf tissue, had a distinct retention time of 15.4 min and showed recoveries of 85% - 90%. This showed that both the extraction method and the separating column had acceptable efficiency levels. After running three HPLC sample injections, external sugar standards of sucrose, fructose, glucose and inulin were injected into the HPLC. This was done regularly to check the stability of the column against prior established concentrations and the retention times of the standards. The calibrations of the standards were stored in a reference system file which was recalled and cross-checked as required. If any significant deviations in retention times and concentrations of the standards were noted, recalibration was done before analysis of subsequent assay samples.

2.2.6 Total Free Amino Acids Quantification

The TFAA were extracted from 200 mg freeze-dried leaf tissue according to the method outlined by Dickson (1979); they were quantified using a modified ninhydrin method described by Cocking and Yemm (1954). The leaf samples were ground into a fine powder in liquid nitrogen using a pre-cooled mortar and pestle. Approximately 20 mg NaF was added to minimise the activity of phosphatases. The macerated samples were extracted with 2 mL of methanol : chloroform : water (12 : 5 : 3, v/v/v).

The extracts were pooled and centrifuged for 10 min at 900 x *g*. The contents were separated into two layers after centrifugation. The upper layer (water: density = 1.0) was expected to contain soluble amino acids, lipids, phenols and pigments. The lower layer (chloroform: density = 1.49) was expected to contain starch, cell walls and tissues. The upper layer was aspirated into 50-mL centrifuge tubes. Another 5 mL of the extracting mixture was added in the aspirated contents. A small quantity of charcoal was added to the contents to clarify the supernatant during centrifugation. The contents were centrifuged further for 10 min at 900 x *g*. This procedure was repeated four times until the samples were devoid of all green colouration.

The clear upper layer containing amino acids and other metabolites was extracted with 10 mL of deionised double-distilled water and then separated by centrifugation for 5 min at 900 x *g*. The upper clear layer was aspirated from the lower green layer, pooled into round-bottomed flasks, and then concentrated under maximum vacuum at 35 °C using a rotary evaporator. The concentrate was redissolved in 1.5 mL, and kept at - 4 °C prior to amino acid assaying. All samples were assayed for total amino acids within 2 days of extraction. Total amino acids were assayed using 40-µL aliquots of the extracts. The aliquots were mixed with 225 µL of freshly

prepared ninhydrin (Sigma®, product Cat. No. 1632) solution in 10-mL test tubes. The ninhydrin reagent was prepared by warming 1.25 g of ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid, with stirring. The reagent was stored in the refrigerator at 2 °C. Fresh reagent was prepared each time a set of samples was analysed. Ninhydrin stock solution was kept at 4 °C in the dark and purged with nitrogen to minimise oxidation. The ninhydrin and samples were incubated in a water bath at 90 °C for 10 min. The reacted mixture was cooled in an ice bath before adding 1 mL of propanol : water (1 : 1, v/v). The contents were thoroughly mixed and brought to room temperature, and absorbance was read at 570 nm. The amount of TFAA was determined from a standard curve denoted by the equation [2.2]:

$$Y = 0.2905 + 0.0138X, r^2 = 0.96 \quad [2.2]$$

where Y is the calculated total amino acids in μg measured by the ninhydrin method, X is the absorbance reading at 570 nm, and r^2 is the coefficient of determination. The standard curve was developed using a series of dilutions that were prepared from a 2 mM L-leucine stock standard solution. Thus, TFAA was expressed as L-leucine equivalent in μg g^{-1} leaf dry weight.

Proline. The free proline was, with some modifications, quantified according to the method of Bates (1973). The free proline was extracted from 200 mg of freeze-dried leaf tissue. The samples were macerated and ground in liquid nitrogen before they were homogenised in 10 mL of 3% sulfosalicylic acid (3-carboxy-4-hydroxybenzene sulfonic acid (Sigma®, product No. 5-2130). The homogenates were filtered through Whatman No. 2 filter paper. Aliquots (1 mL) of the homogenates were placed in 10-mL test tubes, and 1 mL each of acid-ninhydrin and glacial acetic acid were added. The mixtures were reacted for 1 h in a water bath at 100 °C before the reaction was terminated in an ice bath. The free proline was extracted with 2 mL toluene by thorough vortexing for approximately 25 sec. The pink-purple chromophore containing toluene was aspirated from the aqueous layer. This was warmed to room temperature (about 21 °C), before absorbance was read at 520 nm with a Varian® 635 spectrophotometer (Varian Techtron PTY, Australia), using toluene for a blank. The proline concentration was calculated from a standard curve developed using L-proline (Sigma®). The standard curve is represented by the following equation [2.3]:

$$Y = 0.112 + 0.143X, r^2 = 0.98 \quad [2.3]$$

where Y is the calculated proline quantity in μg, X is the absorbance at 520 nm and r^2 is the coefficient of determination. The free proline concentrations were expressed in μg g^{-1} leaf dry weight basis.

2.2.7 Total SDS-Soluble Proteins Quantification

The total SDS-soluble proteins (TSP) were extracted from 200 mg freeze-dried leaf samples using the method described by Pino et al. (1995). The samples were thoroughly ground in liquid nitrogen using a pre-cooled mortar and pestle, followed by protein extraction using SDS (sodium dodecyl sulfate, pH 7.8) buffer. The extraction buffer contained 2% (w/v) SDS, 1% (w/v) DTT (dithiothreitol), 60 mM Tris-HCl, 10% (v/v) glycerol, and 1 mM phenylmethylsulfonyl fluoride (PMSF). The contents were centrifuged twice at 13,000 x *g* for 5 min at 4 °C. After centrifugation, the supernatants were transferred to clean tubes and stored at -20 °C until needed for protein quantification.

The protein concentrations were measured using the bicinchoninic acid (BCA) method described by Brown et al. (1989) in the BCA Protein Assay Reagent Kit (Pierce®, Rockford, IL). A series of known protein concentrations was made from a stock solution containing 2 mg mL^{-1} BSA (bovine serum albumin) protein standard. Extracted protein sample aliquots of 20 µL were reacted with 1000 µL of protein assay reagent in test tubes. The assay reagent was prepared by mixing 1 volume of 4% copper (II) sulphate pentahydrate solution with 50 volumes of BCA solution. The contents were incubated for 30 min at 37 °C and cooled to room temperature (about 20 °C). Absorbance was then read at 562 nm and protein concentration was calculated from a standard curve represented by equation [2.4]:

$$Y = 0.04395 + 0.00087X,\ r^2 = 0.97 \qquad [2.4]$$

where Y is the calculated protein quantity in mg g^{-1} leaf dry wt., X is the absorbance at 562 nm and r^2 is the coefficient of determination.

2.2.8 Nitrate Reductase Activity (NRA) Quantification

NRA (EC 1.6.6.2) was assayed and quantified according to the procedure established by Sanderson and Cocking (1964), with some modifications. Twelve leaf discs were sampled at 30 DAP, under dim green light to avoid light-induced NRA. The leaf discs were cored with a 7-mm diameter cork borer, thus making approximately 462 mm^2 of leaf tissue per sample. The samples were placed in labeled test tubes containing 1 mL of double-distilled water. These test tubes were then incubated at 21 °C for 1 hr in the dark, with continuous agitation. The contents were transferred into 50-mL Falcon tubes containing 10 mL of 0.1 M potassium phosphate buffer (pH 7.5) and 0.3 M potassium nitrate. The stock potassium phosphate buffer solution was made by dissolving 14.51 g of K_2HPO_4 and 2.27 g of KH_2PO_4 in 950 mL of double-distilled water, bringing the volume to 1,000 mL mark. The sample contents were incubated at 21 °C for 1 hr in

darkness with continuous agitation. The reactions of the incubated contents were terminated by adding 200 µL of 1.0 M zinc acetate, followed by 1 mL of 95% ethanol.

The treated reaction mixtures were centrifuged for 5 min at 1,000 x *g*. Aliquots (2 mL) of the supernatant were transferred into test tubes containing 1 mL of 1% sulfaniliamide (p-aminobenzene sulfonamide, Sigma®) in 3 N HCl and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma®). The reacted contents were thoroughly mixed and left to stand for 30 min at 25 °C. Optical densities of the solutions (pink colour) were read at 540 nm with a Varian 635 spectrophotometer (Varian Techtron PTY, Australia). Nitrate reductase activity was determined using a standard curve summarised in equation [2.5]:

$$Y = 0.00812 + 13.80357X,\ r^2 = 0.99 \qquad [2.5]$$

where Y is the calculated nitrate reductase activity in µmoles, X is the absorbance at 540 nm and r^2 is the coefficient of determination. NRA was expressed as µmoles g^{-1} leaf dry wt. The 12 assay leaf discs were saved, dried in the oven, and used to estimate the leaf dry weight of each sample in which NRA was expressed.

2.2.9 Experimental Design and Statistical Analysis

The experiment was conducted in three replicates as a split-plot with the two watering treatments as the mainplots and the eight wheat cultivars as the subplots. The data for the tetraploids and hexaploids were analysed separately. The data for DSI, sucrose, glucose, fructose, fructan, TFAA, free proline, TSP and NRA were subjected to two-way analysis of variance (ANOVA) using the PROC GLM model of the Statistical Analysis System (SAS) version 6.08 (1990). The response data for traits were subjected to one-way ANOVA. The mean values were separated by the least significance difference (LSD at $P < 0.05$). The simple correlation coefficients were determined according to the least squares method using the SAS model PROC CORR. The stepwise multiple regression analyses were performed using the SAS model PROC STEPWISE. The stepwise procedure is designed to include in the regression equation only those terms that contribute significantly to the variation in the dependent variable. This objective is achieved in the stepwise regression technique by systematically adding terms, one at a time, to the regression equation, instead of removing terms, singly or jointly, from an initially large equation. An example of the regression is shown in equation [2.6]:

$$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + ... + \beta_k X_k \qquad [2.6]$$

where α is the intercept (i.e., the value of Y when all X's are zeroes), β_i ($i = 1.....k$), the partial regression coefficient associated with the independent variable X_i, represents the amount of

change in Y for each unit change in X_i. Thus, in the multiple linear functional form with k independent variables, there are (k + 1) parameters (i.e., α, β_1, β_2,.....β_k) that need to be estimated. Thus, the stepwise analysis was conducted to determine the independent variable(s) that could best predict drought stress tolerance using DSI as the dependent variable. The quantitative independent variables were sucrose, glucose, fructose, fructan, TFAA, free proline, TSP and NRA.

2.3 RESULTS

RWC. Following an early drought, the RWC values for both the tetraploids and hexaploids were comparable (Tables 2.1a, 2.1b, 2.3a and 2.3b). The ANOVA for both the tetraploid and hexaploid cultivars showed that cultivar, treatment effects and cultivar x treatment interactions were significant ($P < 0.05$) for RWC. In the current study, RWC was used purposely as a measure of leaf water status.

2.3.1 Tetraploid Wheat Cultivars

Results for DSI are given Tables 2.1a and 2.1b. The cultivar responses showed significant effects for DSI. Pelissier had a significantly lower DSI value than Hercules, thus indicating that Pelissier is more tolerant to drought than Hercules, based on the Fischer and Maurer (1978) interpretation of the DSI.

Results for sucrose, glucose, fructose and fructan contents are given in Tables 2.1c and 2.1d. The ANOVA (Table 2.1d) showed that cultivar effects were significant ($P < 0.05$) for sucrose, glucose, fructose and fructan. Treatment effects were significant for glucose, fructose and fructan. The cultivar x treatment interactions were significant for sucrose, glucose, fructose and fructan. The cultivar responses showed significant effects for, sucrose, glucose and fructose. The results show that an early drought had contrasting effects on the tetraploid cultivars. The sucrose content for Pelissier increased significantly, whereas the sucrose content of Hercules decreased. Early drought decreased the glucose content of both tetraploid cultivars, with Pelissier showing a significantly greater decrease of glucose content than Hercules. The results show that early drought caused a significant increase of fructose and fructan content for both cultivars.

Tables 2.2a and 2.2b give results for TFAA, free proline, TSP and NRA. The ANOVA (Table 2.2b) showed significant cultivar effects for TFAA, TSP and NRA. Treatment effects were significant for TFAA, free proline, TSP and NRA. The cultivar x treatment interactions were significant for TFAA, TSP and NRA. The cultivar response to drought was significant for TFAA, TSP and NRA. Early drought induced significant increases of TFAA, free proline, TSP and NRA

in both cultivars. The results show that under drought, NRA for Pelissier increased, but not as much as for Hercules. Pelissier had significantly higher TFAA than Hercules.

2.3.2 Hexaploid Wheat Cultivars

The results showed that, under drought, Pitic 62 (drought-tolerant) had the lowest DSI (Tables 2.3a and 2.3b). The sucrose, glucose, fructose and fructan results (Tables 2.3C and 2.3d) indicate there were significant cultivar, treatment, cultivar x treatment interaction effects, and trait responses to drought for all four traits. Biggar and K. Mbweha had the highest sucrose content. Biggar had the highest increase for sucrose whilst K. Mbweha had no significant increase in sucrose content.

Early drought caused a decrease in glucose content in all cultivars (Table 2.3c). There was an increase in fructose content in the six hexaploid cultivars. Pitic 62 had the lowest fructose increase whilst K. Mbweha had the highest. The early drought caused a significant increase of fructan for all the cultivars. For example, there was a two-fold fructan increase for Pitic 62, Pasa and K. Nyangumi.

Results for the TFAA, free proline, TSP and NRA test are presented in Tables 2.4a and 2.4b. Early drought on the six hexaploid wheat cultivars caused significant increase for the four traits. The ANOVA (Table 2.4b) showed significant cultivar, treatment, and cultivar x treatment for all four traits. The cultivar responses were significant for free proline and NRA.

2.4 DISCUSSION

DSI. According to Fischer and Maurer (1978), cultivars that show relatively low DSI values are considered to be drought-tolerant. The present findings demonstrate that DSI for the tetraploid cultivars obtained under controlled environmental conditions corroborate with the findings of a 4-year field drought study by Clarke et al. (1992) demonstrating that Pelissier had a mean DSI value of 0.95, whereas Hercules had a mean DSI of 1.14. The present results agree with the previous studies that have demonstrated that, under field drought conditions, Pitic 62 (which had relatively low DSI in the present study) is drought-tolerant relative to other cultivars e.g., Thatcher, Inia, Yecora, White Grain, Potam (Clarke et al., 1992; Hurd, 1974). In terms of DSI, Pitic 62 and Biggar in the present study were grouped as the two most drought-tolerant. The current results for Biggar contrast with the report of Baker (1996) who suggested that Biggar was drought sensitive compared to other cultivars tested. However, relative comparisons of drought sensitivity depend on the genotypes used in the comparisons, and Biggar was the only common cultivar in these two separate studies.

Sucrose. Morgan (1984) showed that sucrose is one of the solutes that accumulate and contribute to osmoregulation in wheat plants that are subjected to drought. The present results show that, under drought, Pelissier accumulates more sucrose than does Hercules. The significant cultivar x treatment interaction for sucrose indicates that, under drought, both tetraploid cultivars are affected differently. The present finding agrees with Jenner (1991) who demonstrated that, under common drought conditions, two wheat cultivars can show contrasting accumulation for each of the carbohydrates sucrose, fructose and glucose. One of the objectives of the current study was to determine if sucrose could be used to predict DSI. Our results showed it could not.

Glucose. The current results contrast with those of Jones et al. (1980) who showed that glucose increased when drought was imposed on sorghum and wheat. However, the current results agree with those of Spollen and Nelson (1994) who demonstrated that in cereal plants, glucose could decrease under drought depending on the plant growth stage and the intensity of drought. The significant cultivar x treatment interaction for glucose indicate that the tested cultivars are differentially affected by drought. One of the objectives of the current study was to determine if glucose could be used to predict DSI. Our results showed it could not.

Fructose. The present results agree with Dubois et al. (1990) who demonstrated, using a different set of wheat cultivars, that fructose was increased under drought conditions. Also, the study results parallel those of Fry et al. (1993) who showed that drought caused a significant increase of fructose in plant leaf tissue. The role of fructose as an osmoregulation solute can be likened to that of sucrose (Morgan, 1984; Jones et al. (1980) as demonstrated in wheat and sorghum. Also, fructose is a substrate for both sucrose and fructan. One could expect that wheat cultivars that accumulate relatively high levels of fructose under drought conditions are likely to accumulate more sucrose and fructan.

From a plant breeder's perspective, it is expected that genotypes that have a relatively high fructose content under drought would have relatively high osmoregulation a benefit for wheat growing under drought as demonstrated by Morgan (1984). Breeders may be able to select for high fructose genotypes given that a significant cultivar x treatment interaction was determined in the present study. One of the objectives of the current study was to determine if fructose could be used to predict DSI. Our results showed it could not.

Fructan. The current results agree with Virgona and Barlow (1991) who used one wheat cultivar (Sun 9), and reported that nonstructural carbohydrates, especially fructan, increased threefold under a drought condition at the Haun 7.+ growth stage (Haun, 1973). It is also assumed that, under drought, the accumulation of fructan may be a reflection of a reduction in sugar

demands. Such decreased sugar demands may result, for example, from decreased growth. This thought is supported by studies conducted by Pollock and Cairns (1991) who demonstrated that the accumulation of fructan in wheat cultivars is partly a result of reduced growth. In the current study, plants under drought conditions were shorter than under non-drought conditions, thus indicating drought caused a decrease of plant growth.

It is possible that, under drought, wheat genotypes that accumulate relatively large amounts of fructan will thrive better than those that accumulate relatively low levels of fructan. According to Dubois et al. (1990), fructan is the main form of reserve carbohydrate for wheat. Thus, cultivars that can accumulate more fructan are most likely to start off with higher carbohydrate reserves once a given drought period is over. One of the objectives of the current study was to determine if fructan could be used to predict DSI. Our results showed it could not.

Total free amino acids (TFAA). The observed increase of TFAA after exposing the tetraploid and hexaploid cultivars to drought corroborates a previous report (Morgan, 1984) which showed that TFAA do accumulate under drought conditions. Previously, Jones et al. (1980) demonstrated, using sunflower and sorghum genotypes that, under drought, TFAA increase and contribute to osmoregulation. Eventually, this may contribute to more grain yield under drought conditions as described by Morgan (1984). In the current study, the important aspect to the breeder is whether the increase of TFAA can predict drought tolerance. This objective was not achieved in our study where drought tolerance was defined on the basis of DSI.

Free proline. In the present study, it was found that proline significantly increased in wheat cultivars after imposing early drought. This is in agreement with a previous study by Martin et al. (1993). Their study, using four wheat cultivars, demonstrated that proline accumulation increased differentially amongst cultivars under drought conditions. Ashraf et al. (1991) used two cultivars, DS-4 (tolerant) and DS-17 (susceptible), and showed that the accumulation of proline was dependent on cultivar, age of the plants and intensity of the drought. In their study, the tolerant cultivar accumulated more proline than the susceptible cultivar. This contrasts with our findings. For example, under drought conditions, Pitic 62, reputed to be drought-tolerant (Clark and Richards, 1988; Hurd, 1974), and Biggar had relatively low free proline.

The lack of differential cultivar (e.g., tetraploids) response to drought for proline in the current study could be a result of several factors. The two tetraploids that are well characterised for drought tolerance and used in the current study did not show any significant differences for proline. It is possible that the two cultivars are not genetically different for proline or the intensity

of the drought that was used did not reach the threshold to differentiate between the two cultivars. This does not exclude the possibility that there are other durums that can show significant differences for proline response.

Under drought, all cultivars in the current study showed a large increase in proline. Possibly, proline has an adaptive role to drought in both tolerant and susceptible cultivars. In other literature (Joyce et al., 1992; Morgan, 1984) proline has been reported as a non-toxic solute for osmoregulation and source for carbon, nitrogen and energy.

For plant breeding purposes, the most important aspect is how or whether responses of proline can be used as a predictor of DSI. In the present study, proline was not a significant predictor of DSI.

Total SDS-Soluble proteins (TSP). The results of the present study agree with those of Hsiao (1970) who indicated that when plants are treated with intervals of drought, they tend to accumulate proteins. The cultivars in the present study were pre-treated with mild drought, re-watered and treated to a second severe cycle of drought before TSP were assayed. Thus, the observed increase of TSP suggests that drought caused the hardened plants to increase proteins.

Under drought, a wide range of plant species including wheat and maize increase their protein content (Pelah et al., 1995). The current study showed that although drought caused an increase of TSP for tetraploid cultivars there was no cultivar effect, or cultivar x treatment interaction effect for TSP.

In contrast, the hexaploids showed significant cultivar x treatment interaction for the TSP. This interaction was substantially contributed by Pasa that displayed relatively low TSP under non-drought conditions, but had the highest response to drought for TSP, and Pitic 62 that had the lowest TSP response to drought. The cultivar x treatment interaction indicates that there are prospects that breeders can find genetic variability for TSP. Higher plants (e.g., wheat) accumulate proteins that serve to protect cell membranes and also protect other proteins from desiccation (He et al., 1995). The proteins are also known to be hydrophilic and serve as part of the solutes for osmoregulation (Pelah et al., 1995). According to one of the objectives of the present study, it was found TSP could not be used for predicting DSI.

Nitrate reductase. There was a relatively higher NRA increase for some cultivars (e.g., Hercules) than others (e.g., Pelissier) under drought. The results confirm studies by Feil et al. (1993) who showed that drought can cause a significant increase in NRA in higher plants, such as wheat and maize.

The present NRA results suggest Pelissier (T) and Hercules (S) differ in the regulation of NRA, or the enzymes from both cultivars are affected differently at a given drought level. Significant cultivar x treatment interaction for tetraploid and hexaploid cultivars imply that there are genetic differences responsible for the NRA variations. Further research is needed to confirm whether the two cultivars differ in genes that regulate the NRA.

The current results shows the six hexaploid cultivars could be classified into two broad groups: (1) low NRA that included Pitic 62, Biggar, and Kwale, and (2) high NRA that included K. Mbweha, K. Nyangumi and Pasa. The low NRA observed in the group 1 cultivars implies that the NR enzymes in this group are not affected by drought as compared to the group 2 cultivars.

Other studies have shown that low NRA cultivars (e.g., Anza, Centurk) rank amongst the top-yielding wheats compared to high NRA cultivars (e.g., Atlas 66) across a wide range of environments (Gallagher et al., 1980). Lines showing low NRA will be desirable, and such lines will not be difficult to identify because NRA is controlled by major genes (Gallagher et al., 1980). However, according to the objective of the present study, it was found NRA could not be used for predicting DSI.

Correlations with DSI. Evaluating whether DSI was significantly correlated with any of the traits listed in Table 2.5 was one of the main objectives of the present study. None of the assayed traits showed any significant correlation with DSI. This result contrasts with a previous study by Dib et al. (1994) who used 25 wheat genotypes that included landraces and improved cultivars from the Mediterranean region and found that under field drought conditions there was a significant correlation (r = - 0.77) between DSI and other traits, such as proline content.

The present results demonstrate that none of the assayed variables could be used to predict DSI. According to Aggrey et al. (1995), response for a single or multiple trait(s) to a given stress depends on genetic variability, intensity of the stress and population size. Of these several factors, population size has the widest range of consequences. In practice, handling large numbers of lines where assaying for a large number of traits is involved may not be economically feasible and breeding progress may become slow if the involved traits have low heritabilities.

For various traits one can get different correlation results amongst studies. According to Aastveit and Aastveit (1993), there are several causes for correlations amongst traits. The causes may include: pleiotropy, linkage, and environmental effects. If pleiotropy or linkage is present, gains could be made by selecting for correlated traits. Individual correlated traits of drought tolerance, for example, may respond to environmental effects with or without changes in other components producing a compensating effect. The influence of pleiotropy or linkage, and

environmental factors, can determine the presence of correlation amongst traits. Morgan (1984) elucidated some of the wheat plant and environmental attributes that can affect traits, such as osmoregulation. The attributes include preconditioning of the plants, age of the tissue assayed, ambient CO_2 concentration, level of drought, photosynthetic active radiation, time of assaying and shading of plants. Thus, it seems there is a wide range of factors that can blend with the range of genetic variability in determining whether one can attain or not attain significant correlations amongst traits.

2.5 Summary and Conclusion

One of the objectives of the current study was to determine whether there were differential responses to early drought when wheat plants were at the Haun 7.+ growth stage (Haun, 1973) for sucrose, glucose, fructose, fructan, free proline, TSP (total SDS-soluble proteins), TFAA (total free amino acids) and NRA (nitrate reductase activity).

Tetraploids. For the two well-characterised tetraploid cultivars Pelissier (tolerant, low DSI) and Hercules (susceptible, high DSI) the following were found:

1). Early drought at the Haun 7.+ growth stage (Haun, 1973), in both cultivars caused significant increase of sucrose, fructose and fructan, except Hercules that had a decrease of sucrose. Glucose content decreased in both cultivars. Early drought caused differential cultivar responses for sucrose, glucose, and fructose. The current results confirmed that specific carbohydrates are affected by drought, using a set of previously non-tested wheat cultivars.

2). Our results demonstrate the specific carbohydrates (sucrose, glucose, fructose and fructan) that are affected by drought. There were significant cultivar x treatment interactions for sucrose, glucose and fructose thus indicating that there were cultivar differences that distinctly changed the effect of drought on these carbohydrates. As to whether these cultivar-specific responses can be utilised by wheat breeders to best predict DSI, will require conducting correlation tests using a larger number of durum genotypes or cultivars. In the present study (Table 2.1c), it is possible to have cultivars that have similar total carbohydrates under both non-drought and drought conditions, but have significantly different levels of individual sugars. As demonstrated by Jones et al. (1980), individual sugars may be more important for contributing to osmoregulation than total sugars.

3). The significant cultivar x treatment interactions for TFAA, TSP and NRA indicates that these traits are differentially affected by drought. To determine whether these cultivar specific responses to drought would be useful for predicting DSI would require correlation tests using more durum genotypes. The current results confirmed the findings of Dingkuhn et al. (1991) that, under drought, TFAA in wheat plants increase significantly and could be part of the solutes contributing to osmoregulation. Also, this study confirmed the findings of Gallagher et al. (1980) that there are cultivar differences in NRA. Further research will be required to confirm whether the measurement of nitrate reductase activity would be useful to predict DSI. Testing of a relatively large number of durum genotypes would be necessary to determine this.

4). The results shows that DSI measurements determined under controlled growth room conditions confirm that Pelissier is drought-tolerant (Clarke and Richards, 1988) and Hercules is drought susceptible (Clarke et al., 1991; Hurd, 1974). When the total sugars were computed, the cultivars were not different. However, when total solutes (carbohydrates + TFAA + TSP) were computed Pelissier had more solutes that Hercules. This suggests that Pelissier is a better total solute accumulator than Hercules. There are no previous reports that have estimated this additivity of solutes for the two cultivars.

Hexaploids. Based on the objectives of the present study, the following findings were noted after inducing drought on wheat plants at the Haun 7.+ growth stage (Haun, 1973):

1). No significant predictor for DSI was found amongst the assayed traits. The complete lack of relationship of any of these traits with drought tolerance estimated by DSI was unanticipated, but removed any possibility of identifying any combinations of these traits (by multiple regression) that would better predict DSI, which was an objective of this study. The lack of correlation with DSI underscores the difficulties underlying the assumptions that single traits can be useful in selection for drought tolerance, consistent with the views expressed by Clarke et al. (1991) and Ehdaie et al. (1988). Perhaps some of the reasons for lack of trait correlation with DSI are due to size of cultivar population, growth stage of assay, extent of stress applied, or a combination of these factors.

2). In this study of six hexaploid cultivars it was found that drought at the tillering stage caused significant increases in sucrose, fructose and fructan, and decreased glucose content in some cultivars. The current results demontrated that early drought caused differential cultivar

responses for sucrose, glucose, fructose and fructan. The results demonstrate the specific carbohydrates that are affected by drought, using a set of previously untested wheat cultivars. For example, in a previous drought study (McCaig and Clarke (1982) Pitic 62 had been reported to accumulate large amounts of carbohydrates although the specific carbohydrates involved were not given. In the present study, we went further and demonstrated the specific carbohydrates (sucrose, glucose, fructose and fructan) that are affected by drought. The present results demonstrate there were significant cultivar x treatment interactions for sucrose, fructose, glucose and fructan thus indicating that there were cultivar differences that distinctly altered the influence of drought on these traits. Nevertheless, these cultivar specific responses were not of importance in predicting DSI.

3). The current results show significant cultivar x treatment interaction for total amino acids, free proline, TSP and NRA. Also, it was found that the imposed drought caused an increase for all four traits. Although cultivar specific responses to drought were found, they were not useful in predicting DSI. However, this does not preclude the possibility that at different drought thresholds and a wider range of genetic variability these traits could predict DSI.

In conclusion, the results show there was no expected gain for any of these traits being useful to breeders for predicting DSI, in contrast to numerous publications which proposed the usefulness of these same individual traits for selecting drought tolerance. In the present study, only one drought stress level was used where plants under drought had approximately 73% leaf RWC at the time of sampling and the available water was 0.06 kg kg^{-1} dry wt. growing medium. There are other drought thresholds, perhaps where different plant responses would be detected. Results from this study indicate that individual traits are certainly not universally useful for identification of tolerant germplasm.

Table 2.1a. Mean ± SE values for relative water content (RWC) and drought susceptibility index (DSI) for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	RWC			DSI
Cultivar	Non-drought	Drought	Response	
Pelissier	95.6 ± 0.9	74.1 ± 0.9	- 21.5 ± 0.6	0.84 ± 0.01
Hercules	96.8 ± 0.2	71.0 ± 0.2	- 25.8 ± 0.2	1.29 ± 0.04
Mean	96.2 ± 0.5	72.6 ± 1.2		1.07 ± 0.18

Table 2.1b. Summary of ANOVA for relative water content (RWC) drought susceptibility index (DSI) for two tetraploid wheat cultivars grown under non-drought and drought conditions.

		Trait mean squares
Source of variation	df	RWC
Replicates	2	0.36
Cultivars (C)	1	2.51*
Error (*a*)	2	0.09
Treatment (T)	1	1677.73**
C x T	1	14.02*
Error (*b*)	4	0.14
R^2		0.97
CV (%)		2.09

		Trait response mean squares	
Source of variation	df	RWC	DSI
Replicates	2	1.34	0.03
Cultivars	1	28.04*	0.19*
Error	2	1.02	0.01
R^2		0.88	0.85
CV (%)		10.5	14.8

df = Degrees of freedom.

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

ns Not significant. CV = Coefficient of variation.

Table 2.1c. Mean ± SE for sucrose, glucose, fructose, fructan and total carbohydrates for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	Sucrose ($\mu g\ g^{-1}$ leaf dry wt.)			Glucose ($\mu g\ g^{-1}$ leaf dry wt.)		
Cultivar	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	0.8 ± 0.02	1.3 ± 0.02	+ 0.49 ± 0.03	4.5 ± 0.09	2.4 ± 0.06	- 2.13 ± 0.07
Hercules	2.4 ± 0.08	1.6 ± 0.02	- 0.79 ± 0.03	3.2 ± 0.01	2.8 ± 0.01	- 0.33 ± 0.03
Mean	1.6 ± 0.05	1.5 ± 0.12		3.9 ± 0.13	2.6 ± 0.16	

	Fructose ($\mu g\ g^{-1}$ leaf dry wt.)			Fructan ($\mu g\ g^{-1}$ leaf dry wt.)		
Cultivar	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	4.3 ± 0.34	12.5 ± 0.18	+ 8.2 ± 0.25	29.0 ± 0.77	40.6 ± 0.69	+ 11.6 ± 0.22
Hercules	3.9 ± 0.22	6.2 ± 0.01	+ 2.0 ± 0.17	27.4 ± 0.47	46.1 ± 0.85	+ 19.0 ± 0.84
Mean	4.1 ± 0.16	9.4 ± 0.57		28.2 ±0.65	43.4 ±0.25	

		†Total carbohydrates ($\mu g\ g^{-1}$ leaf dry wt.)		
Cultivar		Non-drought	Drought	Response
Pelissier		38.6	56.8	+ 18.2
Hercules		36.9	56.7	+ 19.9
	Difference	**	ns	**

†Total sugars = summation of : sucrose, glucose, fructose and fructan.

Table 2.1d. Summary of ANOVA for sucrose, glucose, fructose and fructan for two tetraploid wheat cultivars grown under non-drought and drought conditions.

Source of variation	df	Trait mean squares			
		Sucrose	Glucose	Fructose	Fructan
Replicates	2	0.1	0.1	0.4	3.4
Cultivars (C)	1	4.3**	0.5**	34.7**	13.9*
Error (*a*)	2	0.02	0.01	0.03	0.8
Treatments (T)	1	0.1ns	4.4**	8.1 **	697.7**
C x T	1	2.3**	2.5**	27.0**	41.4*
Error (*b*)	4	0.01	0.03	0.08	3.2
R^2		0.94	0.95	0.94	0.91
CV (%)		5.4	4.9	4.3	8.1

Source of variation	df	Trait responses mean squares			
		Sucrose	Glucose	Fructose	Fructan
Replicates	2	0.01	0.03	0.26	24.54
Cultivars	1	0.03*	4.86**	54.00**	82.88**
Error	2	0.002	0.07	0.02	0.88
R^2		0.94	0.79	0.96	0.81
CV (%)		4.5	20.7	2.7	19.5

df = Degrees of freedom.
CV = Coefficient of variation.
*,** Significant *F*-test at $P < 0.05$ and $P < 0.01$, respectively.
ns Not significant.

Table 2.2a. Mean ± SE values for total free amino acids, free proline, total SDS-soluble proteins and nitrate reductase activity for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	Total free amino acids (TFAA) ($\mu g\ g^{-1}$ leaf dry wt.)			Free proline ($\mu g\ g^{-1}$ leaf dry wt.)		
Cultivar	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	84.8 ± 2.6	118.9 ± 4.1	+ 34.1 ± 2.4	4.6 ± 0.0	41.4 ± 0.1	+ 36.8 ± 0.2
Hercules	87.6 ± 4.6	107.0 ± 3.5	+ 19.4 ± 2.6	4.5 ± 0.0	42.2 ± 0.3	+ 37.5 ± 0.3
Mean	86.2 ± 1.1	112.9 ± 4.9		4.6 ± 0.1	41.8 ± 0.3	

	Total SDS-soluble proteins (TSP) ($mg\ g^{-1}$ leaf dry wt.)			Nitrate reductase activity (NRA) ($\mu moles\ g^{-1}$ leaf dry wt.)		
Cultivar	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	9.8 ± 0.3	13.7 ± 0.3	+ 3.9 ± 0.6	11.4 ± 0.5	18.9 ± 2.2	+ 7.4 ± 1.8
Hercules	10.1 ± 0.5	12.3 ± 0.2	+ 2.2 ± 0.4	12.1 ± 0.0	34.9 ± 2.2	+ 22.8 ± 2.1
Mean	10.0 ± 0.1	13.0 ± 0.6		11.8 ± 0.3	26.9 ± 0.5	

	†Total solutes ($\mu g\ g^{-1}$ leaf dry wt.)		
Cultivar	Non-drought	Drought	Response
Pelissier	221.4	312.7	+ 91.3
Hercules	225.5	286.7	+ 62.3
Difference	*	**	**

†Include total carbohydrates (Table 2.1c), TFAA and TSP. Multiply TSP mean values by 10 before checking the summation of solutes.

Table 2.2b. Summary of ANOVA for total free amino acids, free proline, total SDS-soluble proteins and nitrate reductase activity for two tetraploid wheat grown under non-drought and drought conditions.

		Trait mean squares			
Source of variation	df	Total free amino acids (TFAA)	Free proline	Total SDS-soluble proteins (TSP)	Nitrate reductase activity (NRA)
Replicates	2	43.7	0.1	0.6	4.8
Cultivars (C)	1	62.6*	0.4^{ns}	0.9*	210.0**
Error (*a*)	2	2.5	0.3	0.04	3.9
Treatments (T)	1	2144.0*	4132.9**	28.2**	697.7**
C x T	1	161.3*	0.4^{ns}	2.1*	176.3**
Error (*b*)	4	5.5	0.3	0.2	5.9
R^2		0.92	0.99	0.92	0.87
CV (%)		7.2	1.1	7.1	12.6

		Trait response mean squares			
Source of variation	df	Total free amino acids (TFAA)	Free proline	Total SDS proteins (TSP)	Nitrate reductase activity (NRA)
Replicates	2	68.4	0.1	0.9	6.1
Cultivars	1	321.2**	0.6^{ns}	4.3*	352.7*
Error	2	13.7	0.2	0.2	17.8
R^2		0.76	0.78	0.76	0.72
CV (%)		23.8	21.3	23.9	27.9

df = Degrees of freedom.
CV = Coefficient of variation.
*,** Significant *F*-test at $P < 0.05$ and $P < 0.01$, respectively.
^{ns} Not significant.

Table 2.3a. Mean values for relative water content (RWC) and drought susceptibility index (DSI) for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Cultivar†	RWC			DSI
	Non-drought	Drought	Response	
Pitic 62	96.8 ab‡	74.5 ab	- 22.3 bc	0.54 e
Biggar	96.2 ab	76.7 a	- 19.5 bc	0.69 d
Kwale	92.2 b	76.3 a	- 15.9 c	0.78 c
K. Mbweha	98.9 a	71.4 ab	- 27.5 ab	0.94 b
K. Nyangumi	92.4 b	71.1 ab	- 21.3 bc	0.95 b
Pasa	98.2 a	70.0 b	- 28.2 a	1.05 a
Mean	95.8 ± 1.7	73.3 ± 1.7		0.83 ± 0.1

† Cultivars are listed in the order of DSI.
‡Means within a column followed by the same letter are not significantly different ccording to LSD ($P < 0.05$).

Table 2.3b. Summary of ANOVA for relative water content (RWC) and drought susceptibility index (DSI) for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Source of variation	df	Trait mean squares
		RWC
Replicates	2	0.63
Cultivars (C)	5	16.21 *
Error (*a*)	10	4.12
Treatments (T)	1	4535.57 **
C x T	5	33.62 **
Error (*b*)	12	5.02
R^2		0.95
CV (%)		3.7

Source of variation	df	Trait response mean squares	
		RWC	DSI
Replicates	2	12.38	0.25
Cultivars	5	66.49*	2.49**
Error	10	12.54	0.04
R^2		0.78	0.89
CV (%)		20.9	11.0

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively. df = degrees of freedom.
ANOVA = Analysis of variance. CV = Coefficient of variation.

Table 2.3c. Mean values for sucrose, glucose, fructose and fructan for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Cultivar[†]	Sucrose (μg g^{-1} leaf dry wt.)			Glucose (μg g^{-1} leaf dry wt.)		
	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	1.6 d[‡]	2.9 bc	+1.3 b	4.3 b	1.6 c	- 2.7 ab
Biggar	1.2 e	4.1 a	+2.8 a	3.0 c	0.8 d	- 2.3 ab
Kwale	2.0 c	2.8 bc	+0.8 c	5.9 a	3.0 a	- 2.9 a
K. Mbweha	4.2 a	4.3 a	+0.1 d	3.6 bc	1.9 b	- 1.7 bc
K. Nyangumi	2.7 b	3.1 b	+0.5 c	4.0 bc	3.0 a	- 0.9 c
Pasa	1.9 c	2.5 d	+0.6 c	3.3 bc	0.4 e	- 2.9 a
Mean	2.3 ± 0.6	3.2 ± 0.4		4.0 ± 0.5	1.7 ± 0.6	

Cultivar	Fructose (μg g^{-1} leaf dry wt.)			Fructan (μg g^{-1} leaf dry wt.)		
	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	4.6 b	5.6 d	+0.9 d	30.4 b	73.5 a	+43.1 a
Biggar	2.0 c	6.1 c	+4.1 c	38.6 a	63.7 b	+25.1 c
Kwale	1.1 e	4.9 e	+3.8 c	19.3 c	25.1 d	+ 5.8 e
K. Mbweha	6.2 a	15.7 a	+9.4 a	19.8 c	24.5 d	+ 4.7 e
K. Nyangumi	1.7 cd	3.1 f	+1.3 d	30.9 b	66.9 b	+36.0 b
Pasa	1.3 de	7.3 b	+6.1 b	16.2 c	45.9 c	+29.7 bc
Mean	2.8 ± 1.2	7.1 ± 2.5		25.8 ± 5.0	49.9 ± 12.4	

[†]Cultivars are listed in the order of DSI in Table 2.3a.
[‡]Means followed by the same letter are not significantly different according to LSD ($P = 0.05$).

Table 2.3d. Summary of ANOVA for sucrose, glucose, fructose and fructan for six hexaploid wheat cultivars grown under non-drought and drought conditions.

		Trait mean squares			
Source of variation	df	Sucrose	Glucose	Fructose	Fructan
Replicates	2	0.1	0.1	0.5	11.8
Cultivars (C)	5	3.5**	5.9**	57.6**	2222.6**
Error (*a*)	10	0.9	0.6	6.7	31.9
Treatments (T)	1	9.1**	44.9**	164.3**	8491.6**
C x T	5	1.5**	0.9**	15.0**	820.5**
Error (*b*)	12	0.2	0.1	2.4	23.8
R^2		0.95	0.85	0.95	0.92
CV (%)		4.2	14.6	4.7	6.2

		Trait response mean squares			
Source of variation	df	Sucrose	Glucose	Fructose	Fructan
Replicates	2	0.03	0.10	0.17	7.39
Cultivars	5	2.84**	1.89**	30.25**	1640.57**
Error	10	0.03	0.04	0.11	13.24
R^2		0.83	0.70	0.91	0.86
CV (%)		16.3	28.3	7.8	11.8

df = Degrees of freedom.
CV = Coefficient of variation.
** Significant *F*-test at $P < 0.05$.
ns Not significant.

Table 2.4a. Means values for total free amino acids, free proline, total SDS-soluble proteins and nitrate reductase activity for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Cultivar†	Total free amino acids (TFAA) (μg g^{-1} leaf dry wt.)			Free proline (μg g^{-1} leaf dry wt.)		
	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	113.7 bc‡	130.3 ab	+ 16.5 b	4.5 c	38.1 d	+ 33.5 c
Biggar	99.8 bcd	142.7 a	+ 42.9 ba	7.3 b	37.6 d	+ 30.3 e
Kwale	120.1 ab	143.9 a	+ 23.8 b	7.1 b	39.2 c	+ 32.1 d
K. Mbweha	95.2 cd	134.0 b	+ 38.8 ba	4.5 c	39.2 c	+ 34.6 b
K. Nyangumi	117.8 b	142.1 a	+ 24.3 b	10.2 a	41.9 a	+ 31.7 d
Pasa	83.8 d	138.4 a	+ 54.6 a	4.5 c	40.1 b	+ 35.5 a
Mean	105.1 ±8.3	138.5 ± 3.1		6.4 ± 1.3	39.4 ± 0.9	

Cultivar†	¶Total SDS-soluble proteins (TSP) (mg g^{-1} leaf dry wt.)			¶Nitrate reductase activity (NRA) (μmoles g^{-1} leaf dry wt.)		
	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	13.1 bc‡	14.9 b	+ 1.8 b	14.6 ab	25.7 c	+ 11.1 c
Biggar	11.5 bcd	16.4 a	+ 4.9 ab	13.5 b	15.9 d	+ 2.4 d
Kwale	13.8 ab	16.5 a	+ 2.9 b	8.7 c	18.7 cd	+ 10.0 c
K. Mbweha	10.9 cd	15.4 a	+ 4.4 ab	12.5 b	63.5 a	+ 51.0 a
K. Nyangumi	13.5 ab	16.3 a	+ 2.8 b	6.6 c	53.1 b	+ 46.5 a
Pasa	9.6 d	15.9 a	+ 6.2 a	18.4 a	51.9 b	+ 33.5 b
Mean	12.1 ± 0.9	15.9 ± 0.3		12.3 ± 2.4	38.1 ± 11.7	

†Cultivars are listed in the order of DSI in Table 2.3a.

‡ Means within a column followed by the same letter are not significantly different according to LSD ($P = 0.05$).

Table 2.4b. Summary of ANOVA for total amino acids, free proline, total SDS-soluble proteins and nitrate reductase activity for six hexaploid wheat cultivars grown under non-drought and drought conditions.

		Trait mean squares			
Source of variation	df	Total amino acids	Free proline	Total SDS proteins	Nitrate reductase
Replicates	2	99.9	0.1	1.3	14.7
Cultivars (C)	5	404.4**	16.9**	5.3*	1093.1**
Error (*a*)	10	21.6	3.2	1.4	30.9
Treatments (T)	1	5801.4**	9794.4**	76.6**	6762.3**
C x T	5	1166.9**	6.0**	15.4**	1096.2**
Error (*b*)	12	12.8	0.8	0.9	23.4
R^2		0.89	0.99	0.89	0.87
CV (%)		10.1	0.9	10.0	12.8

		Trait responses mean squares			
Source of variation	df	Total amino acids	Free proline	Total protein	Nitrate reductase
Replicates	2	25.13	0.04	0.34	14.12
Cultivars	5	558.09*	11.60**	7.33*	2189.11**
Error	10	38.06	0.09	2.15	24.33
R^2		0.76	0.85	0.75	0.81
CV (%)		24.5	15.0	24.4	17.9

df = Degrees of freedom.
CV = Coefficient of variation.
*,** Significant *F*-test at $P < 0.05$ and $P < 0.01$, respectively.
ns Not significant.

Table 2.5. Simple correlation coefficients between various pairs of traits for six hexaploid wheat cultivars grown under non-drought and drought conditions.

	DSI		
Trait	Non-drought	Drought	Response
Sucrose	0.02	0.22	- 0.61
Glucose	- 0.30	- 0.30	- 0.61
Fructose	0.04	0.18	0.27
Fructan	0.04	- 0.06	- 0.51
TFAA	- 0.07	0.21	0.01
Free proline	- 0.23	- 0.07	- 0.05
TSP	- 0.09	0.21	0.04
NRA	- 0.23	0.13	0.66

No significant correlations (r, n = 6) were determined at $P < 0.05$.
DSI = Drought susceptibility index. TFAA = Total free amino acids.
NRA = Nitrate reductase activity.
TSP = Total SDS-soluble proteins

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CHAPTER 3

DROUGHT-INDUCED CHANGES IN ELECTROLYTE LEAKAGE, CHLOROPHYLL CONTENT AND PHOTOSYNTHETIC EFFICIENCY IN WHEAT CULTIVARS

3.1 INTRODUCTION

Increasing drought tolerance is one of the major aims of wheat breeding programmes in dry regions. Drought-tolerant cultivars can be described on the basis of drought susceptibility index (DSI) as calculated by Fischer and Maurer (1978). The cultivars showing relatively low DSI are regarded to be drought-tolerant. Several studies on wheat genotypes under field conditions have demonstrated the use of DSI for identifying tolerant cultivars (e.g., Bruckner and Frohberg, 1987; Ceccarelli et al., 1987; Clarke et al., 1992). The DSI was used to estimate relative stress injury because it accounts for variation in yield potential and stress intensity (Bruckner and Frohberg, 1987).

Leaf relative water content (RWC) is accepted as a reproducible and meaningful index of plant water status (McCaig and Romagosa, 1989). In the present study, leaf RWC was used as a measure of leaf water status as described in Chapter 2.

Under drought conditions, measurement of electrolyte leakage (EL) can indicate the critical role of cell membrane stability as a major component of tolerance (Blum and Ebercorn, 1981; Leopold, 1990). EL was used in a drought and heat tolerance study by Blum and Ebercorn (1981) who were able to demonstrate significant differences for EL amongst 13 wheat cultivars. EL increases under , as described by Navari-Izzo et al. (1993) in a study of a single wheat cultivar (cv. Adamello). Elsewhere, Blum (1988, 1989) has suggested that whilst significant and large genetic variation may exist for membrane stability under drought conditions in several crop species (including wheat), the demonstration of an association of EL with drought tolerance at higher plant-organisation levels remains a challenge.

Drought can also adversely affect chlorophyll content and photosynthesis (Lin et al., 1985; Watanabe et al., 1994). Drought can induce reduction in chlorophyll *a* (Chl *a*) with little effect on chlorophyll *b* (Chl *b*) as demonstrated in a field survey using 12 wheat genotypes (Watanabe et al., 1994). Their results showed that, under field drought conditions, wheat cultivars varied significantly for Chl *a*, suggesting that this trait is genetically controlled.

Photosynthetic efficiency (PSE) can be determined from chlorophyll fluorescence that is mainly emitted by Chl *a* and this has been used as an *in vivo* probe for determining drought effects on wheat (Bolhar-Nordenkampf et al., 1989; Di Marco et al., 1988). Also, PSE has been

reported as probe for comparing damage caused by drought amongst 25 durum wheat landraces and improved cultivars derived from the Mediterranean region (Dib et al., 1994). Their studies were conducted both in the field and under controlled conditions and demonstrated that PSE was correlated (r = 0.62*) with DSI. In the meantime, there has been a surge of papers about the application of PSE analysis, particularly for the assessment of photosynthesis in plants under various types of environmental stress (e.g., Mohammed et al., 1995; Schreiber et al., 1995). Most of this work has been on leaves and isolated chloroplasts of species such as pine, poplar and tobacco. A few researchers have applied PSE analysis to wheat in the context of identifying drought-tolerant cultivars (e.g., Dib et al., 1994; Di Marco et al., 1988).

Most studies on EL, chlorophyll content and PSE have been conducted on only one variable at a time, under different drought protocols and without relating the results to DSI. For the purposes of developing breeding strategies there is inadequate information about the relative usefulness of EL, Chl *a*, Chl *b*, Chl T (total chlorophyll) and PSE responses to drought when determined for a range of wheat cultivars under a common drought protocol. If EL, Chl *a*, Chl *b*, Chl T and PSE could be used to predict DSI, under similar controlled drought protocol this might accelerate progress in breeding.

Hypothesis. The hypothesis of the study was that there would be significant association between one or more of the traits electrolyte leakage, Chl *a*, Chl *b*, Chl T and PSE with DSI, and that one or more of these traits would predict DSI.

Objectives. The objectives of this study were to: (1) investigate if there were differential responses to drought for electrolyte leakage, chlorophyll content, and PSE amongst eight spring wheat cultivars of diverse genetic background when evaluated under common drought protocol, and (2) evaluate if cultivar responses to drought of the listed traits under drought can be used to predict drought tolerance based on DSI, including individual and multivariate association with DSI.

The objectives were achieved by conducting concurrent assays of EL, Chl *a*, Chl *b*, Chl T and PSE at 30 DAP (days after planting). These assays were conducted by sampling from plants of each cultivar at a similar Haun 7.+ growth stage (Haun, 1973).

3.2 MATERIALS AND METHODS

3.2.1 Drought susceptibility index (DSI)

A separate experiment using a common drought protocol was used to derive the DSI values (Fischer and Maurer, 1978). This was performed to avoid autocorrelation of data. Some details on how DSI was derived are described in Chapter 2. Briefly, after 30 DAP the drought-

stressed plants were watered similar to the non-drought conditions. In the non-drought treatment, available water was maintained at 0.18 kg water kg^{-1} dry wt. growing medium throughout the experiment, until physiological maturity. At physiological maturity, grain yield was measured and used for computing DSI using the equation [1.1] described in Chapter 1, Section 1.1.7.1.

3.2.2 Cultivars

Two tetraploid and six hexaploid wheat cultivars were evaluated for drought response. Some details of the cultivar materials in regard to pedigree/parentage are listed in Chapter 2, Table 2.0. The tetraploids were selected to represent cultivars of contrasting drought tolerances. The hexaploid wheat cultivars were also chosen to represent a range of drought tolerance, based on literature reports, genetic diversity, pedigree and morphological differences. There are no records showing how the listed hexaploid cultivars respond to drought in terms of EL, PSE and Chl content.

3.2.3 Plant Growth Conditions and Leaf Assays

The details of plant growth conditions and leaf RWC were described in Chapter 2. The youngest fully emerged and expanded leaves (YFEEL) derived from four plants were used for the assays of EL, Chl *a*, Chl *b*, Chl T and PSE. All leaf samples were assayed at 30 DAP when the plants had developed a similar Haun 7.+ growth stage (Haun, 1973). This growth stage was consistently used in all assays to reduce differences that would be caused by variability in leaf phenological stages.

3.2.4 Electrolyte Leakage

The EL was measured using 12 discs of leaf tissue (~ 462 mm^2). The leaf discs were cored from the YFEEL using a 7-mm diameter cork borer. Throughout the experiment the youngest and fully emerged leaves of the main stems were sampled from test cultivars. This was done to minimise variations attributed to leaf phenological stages as outlined by Clarke (1992). Prior to making leaf cores, wheat leaf blades were quickly rinsed with double-distilled water to remove any traces of salts or electrolytes remaining on the leaf surface, and blotted to remove any adhered water droplets. The leaf discs were placed in 50-mL Falcon® tubes with caps (Becton Dickson & Co., NJ) containing 10 mL of double-distilled water. The contents were continuously agitated for 24 h at room temperature using a bench-top shaker before initial electrical conductivity was measured using a Radiometer CDM 83 conductivity meter (Bach-Simpson Ltd., London, ON). Total electrolytes were measured after boiling the samples in a microwave oven, cooling the contents for 2 h with shaking, adjusting the volume to 10 mL and re-measuring final

EC. The electrolyte leakage (EL) was calculated according to a protocol described by Tahir and Singh (1993) as in equation [3.1]:

$$EL = [EC_1/EC_2] \times 100 = [1 - [1 - (EC_1/EC_2)]] \times 100 \qquad [3.1]$$

where EC_1 is the initial electro-conductivity, and EC_2 is the final electro-conductivity. In the present study, equation [3.1] was used because of its advantages (e.g., efficiency, accuracy) as outlined by Tahir and Singh (1993).

3.2.5 Chlorophyll Quantification

Chlorophyll was extracted under green dim-light conditions as described by Vernon (1960). Representative leaf samples for each cultivar were obtained from four YFEEL of the main stems at 30 DAP. About 0.5 g of leaf tissue was weighed and macerated in liquid nitrogen using pre-cooled mortar and pestle. During maceration, 10 mg of $CaCO_3$ were added to the leaf tissue to reduce chlorophyll decomposition. The macerated tissues were extracted using 80% ice-cold acetone until tissues were depleted of all green colour. Extracts were filtered through fine miracloth and the filtrate was diluted to a known volume, using 80% cold acetone. The Chl *a*, Chl *b* and Chl T content were measured by the spectrophotometry procedure of Vernon (1960) and computed using the following equations [3.2], [3.3] and [3.4]:

$$\text{Chl } a = (11.63)\, A_{665} - (2.39)\, A_{649} \qquad [3.2]$$

$$\text{Chl } b = (20.11)\, A_{649} - (5.18)\, A_{665} \qquad [3.3]$$

$$\text{Chl T} = (6.45)\, A_{665} + (17.72)\, A_{649} \qquad [3.4]$$

where the numbers in brackets (...) are coefficients, A_{649} is absorbance value at 649 nm and A_{665} is absorbance at 665 nm and the results were expressed in mg g^{-1} leaf dry weight.

3.2.6 Photosynthetic Efficiency (PSE)

The PSE was determined from chlorophyll fluorescence induction that was assayed on the YFEEL from the main stems. Four leaves were used from each of the four plants per cultivar within a replicate, thus making 16 readings per replicate. PSE was measured on the upper surface of the leaf using a Model SF-30 Plant Productivity Fluorometer (Richard Brancker Research Ltd., Ottawa, ON). Fluorescence was induced by using an excitation wavelength of 670 nm (red light), using a light emitting diode. Fluorescence emission was detected at 710 nm. Prior to fluorescence measurements, plants were dark-adapted for 40 min at room temperature (22 °C) before initial fluorescence (*Fo*), and maximum fluorescence (*Fm*) were measured. The data were recorded over 90 s, following 60 s of detached leaf dark adaptation, at an actinic light emission of 80 W m^{-2}. Variable fluorescence (*Fv*) was defined as *Fm* - *Fo*, whilst PSE was defined as (*Fv*/*Fm*) for each cultivar within each water treatment.

3.2.7 Experimental Design and Data Analysis

The experiment was conducted in three replicates in a split-plot design with the two water treatments (non-drought and drought) as the main plots and the eight wheat cultivars as sub-plots. Data for tetraploid and hexaploid cultivars were analysed separately. Data for the measured and calculated variables were subjected to standard statistical analysis, using the Statistical Analysis System 6.08 (SAS, 1990). Means were computed and their differences tested for significance, using the LSD ($P < 0.05$) procedure with the Student's *t*-test. Simple correlation analyses were conducted to determine the association between DSI and other measured variables. The correlation values were computed using the PROC CORR as described in SAS (1990). The stepwise multiple regression analyses were performed using the SAS model PROC STEPWISE described in Chapter 2.

3.3 RESULTS

3.3.1 Tetraploid Wheat Cultivars

Table 3.1a shows that the imposed early drought at similar Haun 7.+ growth stage (Haun, 1973), caused significant increase of EL in the leaves. The ANOVA (Table 3.1b) showed significant cultivar, treatment but no significant cultivar x treatment interaction and response for EL. Results given in Table 3.2a show that early drought caused a significant decrease of Chl *a* in both Pelissier and Hercules. The ANOVA (Table 3.2b) shows that cultivar, as well as treatment effects were significant for Chl *a*. There was no significant cultivar variation in response to drought for Chl *a*. There was no significant cultivar, treatment, cultivar x treatment or cultivar response to drought for Chl *b*. Cultivar, and treatment effects were significant for Chl T, but no significant cultivar x treatment interaction or cultivar response to drought for Chl T were determined.

Table 3.3a shows that drought caused a significant decrease of PSE (i.e., photosynthetic efficiency) for both cultivars. Under drought, Hercules showed a two-fold decrease in PSE relative to Pelissier. The ANOVA (Table 3.3b) showed significant cultivar, treatment, and cultivar x treatment effects as well as cultivar response to drought for PSE.

3.3.2 Hexaploid Wheat Cultivars

The drought tolerance and a description of the six hexaploid cultivars is given in Chapter 2. Tables 3.4a and 3.4b show results for EL. Under drought conditions, Kwale, K. Nyangumi and Pasa showed the lowest level of EL, whereas Pitic 62 showed the highest level of EL despite the low DSI noted for Pitic 62 in Chapter 2. The current results show that Pitic 62 had the highest EL

response and Pasa had the lowest. The ANOVA (Table 3.4b) showed significant cultivar, treatment, cultivar x treatment, and cultivar responses to drought for EL.

Tables 3.5a and 3.5b show results for Chl *a*, Chl *b* and Chl T. Kwale showed the highest negative Chl *a* response and Biggar and Pitic 62 showed the lowest negative Chl *a* response to drought. Under drought, Pitic 62 and Kwale had the lowest Chl *b*. Biggar showed the lowest Chl T response to drought. The ANOVA (Table 3.5b) showed significant cultivar, and treatment variations for Chl *a*, Chl *b*, and Chl T. Cultivar x treatment interactions, and cultivar responses to drought were significant for Chl *a* and Chl T.

The results for PSE (i.e., *Fv/Fm*) in Tables 3.6a and 3.6b show that drought significantly decreased PSE. Pitic 62, Biggar and K. Mbweha showed the lowest PSE response to drought, whereas Kwale and Pasa showed the highest response. The ANOVA showed significant cultivar, treatment, cultivar x treatment, and cultivar response to drought for PSE.

The results in Table 3.7 show that, under non-drought conditions, DSI was positively and significantly correlated with Chl *a* and Chl T. Under drought conditions, DSI was negatively correlated with EL as well as EL response to drought. Under control conditions Chl T was a significant predictor for DSI, whilst under drought conditions EL was a significant predictor of DSI.

3.4 DISCUSSION

EL. In the present results, under drought conditions, both tetraploids and hexaploids showed a significant increase in EL. The results agree with Blum and Ebercorn (1981) who showed that there was a significant increase of EL for 12 wheat cultivars that were tested under field drought conditions and high heat stress conditions. In their study, drought was confounded with heat, whereas in the present study heat was not a factor. The present results indicate that drought caused a similar response in EL for Pelissier and Hercules. Possibly, the drought intensity used in the current study was not severe enough to differentiate the response of the two tetraploid cultivars.

Although EL has been used to differentiate wheat genotypes in terms of tolerance to other thresholds of stresses, such as heat (Blum, 1988) and chilling conditions (Janowiak and Markowski, 1994), its association with drought tolerance has remained unclear. Whereas the basic knowledge of the effect of water deficit on the membrane and the associated responses in terms of EL would support the use of EL as an index of drought tolerance, an association between the level of EL and total plant performance under drought as demonstrated can complex.

The current EL findings suggest, for example, that Pitic 62, Biggar, and Kwale are not tolerant to drought compared with K. Mbweha, K. Nyangumi and Pasa. Interpreting the EL results as such would contrast with the DSI results which show that Pitic 62, Biggar and Kwale are more tolerant than K. Mbweha, K. Nyangumi and Pasa. It was expected that drought would induce lower electrolyte leakage in drought-tolerant cultivar(s), such as Pitic 62 compared with drought susceptible cultivar(s), such as Pasa on the basis of DSI. However, this was not the case. This raises the question as to whether all the observed EL is a result of membrane damage or whether there is interaction with other processes such as osmoregulation.

Morgan (1984) indicated that tolerant wheat cultivars can undergo the process of osmoregulation by increasing solutes during a drought period. According to Morgan (1984, 1991), under drought conditions, wheat cultivars that have relatively high osmoregulation also produce more grain. Based on published literature and findings of the present study, it is thought that although there would have been cell membrane damage, under drought conditions, the relatively high EL determined in the drought-tolerant cultivars e.g., Pitic 62, Biggar and Kwale could be a result of solute accumulation.

It was shown in Chapter 2 that, under drought conditions, solutes including sucrose, fructose, fructan, total amino acids, proline, soluble proteins and nitrates significantly accumulated in both tetraploid and hexaploid cultivars. These solutes also may have contributed to EL increase.

A significant cultivar x treatment interaction for EL has practical implications. It means there is genetic variability for EL on which wheat breeders may be able to base selection.

Under drought conditions, EL was negatively correlated with DSI. This indicates that drought-tolerant cultivars (i.e., those with low DSI) produce more EL than drought susceptible cultivars (i.e., those with high DSI).

The current results suggest that the cultivars with relatively high EL under drought conditions are tolerant to drought based on DSI. This supports the postulation that EL is showing not just the extent of membrane damage, but to a large extent the total solutes increase (e.g., through osmoregulation) that accumulated during the stress period. This inference is supported by the similarity of responses of known drought-tolerant cultivars from unrelated dry land wheat farming systems, in N. America (e.g., cv. Pitic 62, Pelissier) and E. Africa (e.g., cv. Kwale).

Chl *a* . The decrease of Chl *a* under early drought conditions was common for both tetraploid and hexaploid cultivars. The present results agree with Quartacci and Navari-Izzo (1992) who demonstrated that, by withholding water for six days under controlled drought

conditions, Chl *a* was significantly decreased in species such as wheat, sunflower and barley. The present study found that early drought caused a decrease of Chl *a* amongst cultivars. Pitic 62 and Biggar both established to be drought-tolerant according to the literature, and DSI in this study, showed the lowest Chl *a* decrease under drought conditions. The significant cultivar x treatment interaction for Chl *a* in the present study implies that there were genetic differences that were influencing the abundance of Chl *a* depending on the plant growing environmental conditions. Furthermore, this means that wheat breeders may be able to select for Chl a, and its response to drought.

Under non-drought conditions, DSI was positively correlated with Chl *a*. This can be interpreted that wheat cultivars with relatively low Chl *a* are drought-tolerant (i.e., low DSI), whereas cultivars with relatively high Chl *a* are drought susceptible (i.e., high DSI).

Chl *b*. Early drought caused a decrease of Chl *b* the hexaploid wheat cultivars. This finding contrasts with the results of Quartacci and Navari-Izzo (1992), who demonstrated that, under controlled drought conditions, Chl *b* was not significantly decreased in barley, wheat and sunflower plants. However, for the tetraploids the results of the present study agrees with Quartacci and Navari-Izzo (1992). In the current study, the tetraploids showed no cultivar, treatment or cultivar x treatment variations, whereas for the hexaploids there were significant cultivar, and treatment effects.

The lack of response in Chl *b* to drought amongst most of the current test cultivars may be interpreted as a lack of genetic variability specifically for the Chl *b*. Also, it may be that Chl *b* requires a different level of drought intensity and duration before cultivar differences can be detected. The present results indicate that Chl *b* may not be useful in plant breeding for selecting wheat genotypes differing in drought tolerance.

Chl T. Early drought caused a decrease of Chl T for both tetraploid and hexaploid cultivars. The present results corroborate with Quartacci and Navari-Izzo (1992) who demonstrated that drought caused a decrease of Chl T in higher plants such as wheat and sunflower.

In the present study, it was determined that there was a significant cultivar x treatment interaction for Chl T. This suggests that wheat breeders have genetic variability for Chl T from which they can select genotypes under various environments. Austin et al. (1988), using four species of wheat, demonstrated that there was a Chl T x species interaction for maximum photosynthesis. The present results show cultivar x treatment interaction for Chl T and cultivars, such as Pitic 62 and Kwale, that were grouped higher in drought tolerance on the criterion of DSI

did not necessarily show higher Chl T under drought conditions. This suggests that (at least in hexaploid wheat cultivars), relatively high Chl T may not be associated with drought tolerance.

Under non-drought conditions, DSI was positively correlated with Chl T. Thus, wheat cultivars with relatively low Chl T are drought-tolerant (i.e., low DSI), whereas cultivars with relatively high Chl T are drought susceptible (i.e., high DSI). Our finding supports that of Austin et al. (1988) who also demonstrated a correlation between Chl content and adaptation to drought conditions amongst four diploid *Triticum* spp. (landraces from dry environments) and one *T. aestivum* genotype in a greenhouse/laboratory study. Their results demonstrated that the diploid species had a lower chlorophyll content and thinner leaves relative to a *T. aestivum* genotype. Also, Austin et al. (1988) demonstrated that *T. aestivum* had approximately 35% more Chl content than each of the diploid species. However, *T. aestivum* showed significantly lower photosynthetic efficiency compared to the diploid species that had lower Chl content.

This suggests that breeders need to select genotypes with relatively low Chl content, to improve drought tolerance. Under drought conditions, cultivars with high Chl content could trap excess light energy that can not be fully utilised. Such energy may become detrimental through inhibition of cell processes as described by Lawlor (1993). It is possible too that cultivars with relatively high Chl content may suffer other confounding effects such as high internal temperatures. The internal temperatures may rise because of absorbing more light energy in contrast to relatively low Chl content cultivars that have light green leaves and can reflect more light energy, thus maintaining a low internal temperature.

This finding implies that it is possible to select for Chl T under non-drought conditions and attain cultivars that perform well under drought conditions. Richards (1982) and Braun et al. (1992) proposed that selecting high-grain-yielding wheat cultivars targeted for drought prone environments can be achieved by conducting selection under environments with adequate water. They argued that this approach is likely to identify germplasm that could combine high genetic grain yield potential with tolerance to drought contrary to selection conducted exclusively under drought conditions. Our study illustrates and supports this approach from a physiological point that one can select cultivars with low DSI (drought-tolerant) by selecting for relatively lower Chl content. However, what requires more research is how much lower Chl content can be selected for without compromising grain yield potential because of limiting chlorophyll content itself. This research area requires more attention.

PSE. The present results show that, under drought, both the tetraploid and hexaploid wheat cultivars significantly decrease their PSE. Bolhar-Nordenkampf et al. (1989) also

demonstrated that drought decreases PSE; however, this was only in one wheat genotype. The present results show cultivar x treatment interaction for PSE suggesting that there is genetic variability in both species that wheat breeders can exploit.

The measurement of PSE is a non-destructive technique and is being used increasingly to study the effects of stress on photosynthesis. In several species, PSE has been shown to be a sensitive indicator of stresses comprising those caused by lack of water (Di Marco et al., 1988), chilling temperatures (Bertin et al., 1996), and heat (high temperatures) (Srinivasan et al., 1996). Although the literature has shown that there is an association between PSE and tolerance to these stresses, our study did not lend support to the premise that PSE can be a useful predictor of drought tolerance based on DSI.

Most studies on the effects of drought on PSE have been short-term on plants or leaves subjected to relatively rapid dehydration. It is speculated that such studies could give information on the effects of imposed short periods of high evaporative demand. However, they may be of less relevance to droughted crops grown to maturity, where drought increases gradually, allowing physiological and morphological acclimation by the plant as in the present study.

3.5 Summary and Conclusions

The current results demonstrate that imposed early drought, to some degree, negatively affected both the two tetraploid and six hexaploid wheats, for EL, Chl *a*, Chl *b*, Chl T and PSE. The following observations could be made for each species:

Tetraploids. The current results show that one can study wheat cultivars under controlled conditions and obtain drought tolerance information comparable to results obtainable under field conditions. Our results show that characterisation of Pelissier as tolerant and Hercules as susceptible was the same as determined by field studies (Clarke and Richards, 1988; Clarke et al., 1991).

1). There is no published literature regarding EL for Pelissier and Hercules. The present results demonstrate that there was no cultivar x treatment interaction for EL. This indicates that the two cultivars are not significantly different in terms of membrane stability assuming EL is a result of membrane damage as described by Blum and Ebercorn (1981). The two cultivars are similar in response to drought for EL, although both are genetically dissimilar based on pedigree/parentage. It is possible that the two cultivars require a more severe drought threshold than in this study for EL differences to be apparent.

2). There is no prior literature on these cultivars regarding their Chl changes upon imposition of drought. The current results show that the imposed drought on plants that were at a similar Haun 7.+ growth stage (Haun, 1973), caused significant decreases of Chl *a*, Chl T and PSE, but not Chl *b*, and that there was significant cultivar x treatment interaction for Chl *a* and Chl T. These findings could be interpreted as follows. Under differing growing conditions there are genetic factors, depending on the cultivar, that influence the abundance of both Chl *a* and Chl T. These genetic factors, depending on their heritability, could be exploited by wheat breeders. A study by Austin et al. (1988) has shown that Chl content can be manipulated amongst various wheat ploidy levels through backcrossing procedures.

In conclusion, it is possible to extrapolate from the results of this study of two durum cultivars, that Chl content, PSE and DSI could be useful for selecting drought-tolerant durums. A simple approach could be to study the cross of Hercules x Pelissier, and to apply the assay protocols used herein for PSE and DSI in the segregating population. In addition, it could be appropriate to screen a wide germplasm base in durum for further variability in PSE, to see if it could be used as a predictor for new sources of drought tolerance.

Hexaploids. The current results show that induced drought can cause differential effects on hexaploid wheat cultivars at the Haun 7.+ growth stage (Haun, 1973), thus satisfying one of the objectives of the current study.

1). There is no published literature on the six hexaploid cultivars regarding EL changes after being subjected to drought. Induced drought caused significant positive effects in EL, thus suggesting that the six cultivars have varied cell membrane stability under drought conditions (Blum and Ebercorn, 1981). Whether the observed genotypic differences in EL are heritable remains to be researched.

2). Under drought conditions EL can significantly predict DSI. This information is important for wheat breeders as it implies that, one can select for drought-tolerant cultivars by selecting those cultivars with relatively high EL. Under non-drought conditions, Chl *a* or Chl T could be used as a predictors for DSI. Austin et al. (1988) noted that diploid wheat landraces that had relatively low chlorophyll were well-adapted to dry environments, although their study did not link this to DSI.

3). The present results did not show PSE to be a significant predictor of drought tolerance in the tested materials. Perhaps a very severe drought is required before PSE can differentiate cultivar tolerances to drought. This finding confirms the assertions made by others (pers. comm.,

Blum) that "PSE is a small window that cannot be used effectively to determine drought tolerance in wheat cultivars."

In conclusion, the current results demonstrate that induced drought on wheat cultivars at similar Haun 7.+ growth stage (Haun, 1973), caused significant and differential effects on DSI, EL, Chl *a*, and Chl T in six hexaploid cultivars. Our study determined that EL, Chl *a*, and Chl T can be used predictors of DSI. Further research is needed to determine the heritability of these traits in segregating populations and when applied to a wider germplasm base.

Table 3.1a. Mean ± SE values for electrolyte leakage (EL) for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	EL		
Cultivar	Non-drought	Drought	Response
Pelissier	14.1 ± 0.3	22.9 ± 0.1	+ 8.9 ± 0.3
Hercules	17.4 ± 0.2	25.9 ± 0.9	+ 8.5 ± 0.8
Mean	15.8 ± 1.3	24.4 ± 1.2	

Table 3.1b. Summary of ANOVA for electrolyte leakage (EL) for two tetraploid wheat cultivars grown under drought conditions.

		Trait mean squares
Source of variation	df	EL
Replicates	2	1.41
Cultivars (C)	1	30.08 **
Error (*a*)	2	0.53
Treatment (T)	1	227.07 **
C x T	1	0.08 ^{ns}
Error (*b*)	4	0.05
R^2		0.96
CV (%)		3.55

		Trait responses mean squares
Source of variation	df	EL
Replicates	2	0.09
Cultivars	1	0.17 ^{ns}
Error	2	0.06
R^2		0.89
CV (%)		11.3

df = Degrees of freedom.
*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.
^{ns} Not significant. CV = Coefficient of variation.

Table 3.2a. Mean ± SE values for chlorophyll *a*, chlorophyll *b* and total chlorophyll for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	Chlorophyll (mg g^{-1} leaf dry wt.)*a*			Chlorophyll *b* (mg g^{-1} leaf dry wt.)		
†Cultivar	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	1.33 ± 0.04	0.93 ± 0.01	- 0.41 ± 0.05	0.59 ± 0.05	0.48 ± 0.01	- 0.11 ± 0.02
Hercules	1.39 ± 0.01	1.12 ± 0.01	- 0.37 ± 0.03	0.56 ± 0.03	0.44 ± 0.03	- 0.12 ± 0.02
Mean	1.41 ± 0.06	1.03 ± 0.11		0.57 ± 0.05	0.46 ± 0.06	

	Total chlorophyll (mg g^{-1} leaf dry wt.)		
†Cultivar	Non-drought	Drought	Response
Pelissier	1.62 ± 0.01	1.12 ± 0.01	- 0.50 ± 0.03
Hercules	2.26 ± 0.02	1.66 ± 0.02	- 0.60 ± 0.04
Mean	1.94 ± 0.06	1.39 ± 0.04	

Table 3.2b. Summary of ANOVA for chlorophyll *a*, chlorophyll *b* and total chlorophyll for two tetraploid wheat cultivars grown under drought conditions.

Source of variation	df	Trait mean squares		
		Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Replicates	2	0.01	0.01	0.01
Cultivars (C)	1	0.42 **	0.14 ns	1.04 **
Error (*a*)	2	0.002	0.02	0.003
Treatment (T)	1	0.72 **	0.01 ns	0.91 **
C x T	1	0.01 ns	0.01 ns	0.01 ns
Error (*b*)	4	0.007	0.006	0.002
R^2		0.96	0.85	0.95
CV (%)		3.84	12.3	2.48

Source of variation	df	Trait response mean squares		
		Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll
Replicates	2	0.001	0.001	0.004
Cultivars	1	0.008 ns	0.005 ns	0.011 ns
Error	2	0.004	0.003	0.003
R^2		0.81	0.76	0.89
CV (%)		18.9	24.2	9.8

ANOVA = Analysis of variance
df = Degrees of freedom.
** Significant at $P < 0.01$.
ns Not significant.
CV = Coefficient of variation.

Table 3.3a. Mean ± SE values for photosynthetic efficiency (PSE) for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	PSE		
Cultivar	Non-drought	Drought	Response
Pelissier	0.43 ± 0.05	0.31 ± 0.01	- 0.12 ± 0.05
Hercules	0.47 ± 0.07	0.24 ± 0.03	- 0.23 ± 0.06
Mean	0.45 ± 0.02	0.28 ± 0.03	

Table 3.3b. Summary of ANOVA for photosynthetic efficiency (PSE) for two tetraploid wheat cultivars grown under non-drought and drought conditions.

		Trait Mean squares
Source of variation	df	PSE
Replicates	2	0.001
Cultivars (C)	1	0.05 *
Treatment (T)	1	0.09 **
Error (*a*)	2	0.002
C x T	1	0.03 *
Error (*b*)	4	0.001
R^2		0.83
CV (%)		17.2

		Trait response mean squares
Source of variation	df	PSE
Replicates	2	0.02
Cultivars	1	0.18**
Error	2	0.01
R^2		0.85
CV (%)		15.1

df = Degrees of freedom. CV = Coefficient of variation.

*, ** Significant at $P < 0.0$ and $P < 0.05$, respectively.

‡ PSE = photosynthetic efficiency = *Fv/Fm*, where *Fv* = variable and *Fm* = maximum fluorescence.

Table 3.4a. Mean values for electrolyte leakage (EL) for six hexaploid wheat cultivars grown under non-drought and drought conditions.

	EL		
Cultivar †	Non-drought	Drought	Response
Pitic 62	17.1 d	31.7 a	+ 14.6 a
Biggar	15.3 e	27.3 b	+ 12.1 b
Kwale	11.1 f	23.8 c	+ 12.7 b
K. Mbweha	19.0 b	26.8 b	+ 7.8 c
K. Nyangumi	17.9 c	24.0 c	+ 6.1 d
Pasa	21.0 a	24.0 c	+ 3.0 e
Mean	16.9 ± 1.9	26.3 ± 1.8	

†Cultivars are listed in the order of DSI in Chapter 2, Table 2.3a.

‡Means within a column followed by the same letter are not significantly different according to LSD ($P < 0.05$).

Table 3.4b. Summary of ANOVA for electrolyte leakage (EL) for six hexaploid wheat cultivars grown under non-drought and drought conditions.

		Trait mean squares
Source of variation	df	EL
Replicates	2	0.43
Cultivars (C)	5	33.93 **
Error (*a*)	10	0.28
Treatments (T)	1	790.54**
C x T	5	29.73**
Error (*b*)	12	0.15
R^2		0.98
CV (%)		1.8
		Trait response mean squares
Source of variation	df	EL
Replicates	2	0.11
Cultivars	5	55.86**
Error	10	0.29
R^2		0.94
CV (%)		5.6

ANOVA = Analysis of variance. df = Degrees of freedom. CV = Coefficient of variation.
*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 3.5a. Mean values for chlorophyll *a*, chlorophyll *b*, and total chlorophyll drought for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Cultivar†	Chlorophyll *a* (mg g^{-1} leaf dry wt.)			Chlorophyll *b* (mg g^{-1} leaf dry wt.)		
	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	1.25 c‡	1.12 c	- 0.13 d	0.47 b	0.39 b	- 0.07 ab
Biggar	1.47 ab	1.39 a	- 0.08 d	0.55 ab	0.52 a	- 0.03 b
Kwale	1.39 b	0.89 d	- 0.50 a	0.49 ab	0.38 b	- 0.11 a
K. Mbweha	1.51 a	1.08 c	- 0.43 ab	0.57 ab	0.53 a	- 0.04 b
K. Nyangumi	1.52 a	1.12 c	- 0.39 b	0.56 ab	0.53 a	- 0.03 b
Pasa	1.56 a	1.29 b	- 0.30 c	0.60 a	0.56 a	- 0.04 b
Mean	1.45 ± 0.06	1.14 ± 0.10		0.54 ± 0.02	0.48 ± 0.04	

Cultivar	Total chlorophyll (mg g^{-1} leaf dry wt.)		
	Non-drought	Drought	Response
Pitic 62	1.72 c	1.51 d	- 0.21 d
Biggar	2.00 ab	1.95 a	- 0.05 e
Kwale	1.88 bc	1.26 e	- 0.61 a
K. Mbweha	2.09 a	1.61 cd	- 0.48 ab
K. Nyangumi	2.04 ab	1.68 bc	- 0.36 bc
Pasa	2.16 a	1.83 ab	- 0.32 dc
Mean	1.98 ± 0.09	1.64 ± 0.14	

† Cultivars are listed in the order of DSI in Chapter 2, Table 2.3a.
‡ Means within a column followed by the same letter are not significantly different according to LSD ($P < 0.05$).

Table 3.5b. Summary of ANOVA for chlorophyll *a*, chlorophyll *b* and total chlorophyll for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Source of variation	df	Trait mean squares		
		Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
Replicates	2	0.01	0.01	0.02
Cultivars (C)	5	0.85 **	0.02*	1.04 **
Error (a)	10	0.002	0.003	0.003
Treatments (T)	1	0.08 **	0.04**	0.05 **
C x T	5	0.04 **	0.04*	0.19 **
Error (*b*)	12	0.002	0.004	0.008
R^2		0.95	0.86	0.95
CV (%)		4.1	12.9	5.0

Source of variation	df	Trait response Mean squares		
		Chlorophyll *a*	Chlorophyll *b*	Total Chlorophyll
Replicates	2	0.02	0.001	0.06
Cultivars	5	0.09**	0.008*	0.12 **
Error	10	0.002	0.001	0.01
R^2		0.85	0.85	0.74
CV (%)		14.7	15.0	24.9

ANOVA = Analysis of variance
df = Degrees of freedom.
*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.
CV = Coefficient of variation.

Table 3.6a. Mean values for photosynthetic efficiency (PSE) for six hexaploid wheat cultivars grown in non-drought and drought conditions.

	PSE		
Cultivar†	Non-drought	Drought	Response
Pitic 62	0.59 b‡	0.33 a	- 0.26 c
Biggar	0.58 b	0.29 a	- 0.29 c
Kwale	0.70 a	0.31 a	- 0.39 a
K. Mbweha	0.59 b	0.31 a	- 0.28 c
K. Nyangumi	0.64 ab	0.33 a	- 0.31 bc
Pasa	0.67 a	0.31 a	- 0.36 ab
Mean	0.62 ± 0.02	0.31 ± 0.01	- 0.32 ± 0.03

†Cultivars are listed in the order of DSI in Chapter 2, Table 2.3a.
‡Means within a column followed by the same letter are not significantly different according to LSD ($P < 0.05$).

Table 3.6b. Summary of ANOVA for photosynthetic efficiency (PSE) for six hexaploid wheat cultivars grown under non-drought and drought conditions.

		Trait mean squares
Source of variation	df	PSE
Replicates	2	0.01
Cultivars (C)	5	0.04*
Error (*a*)	10	0.002
Treatments (T)	1	0.89**
C x T	5	0.03*
Error (*b*)	12	0.001
R^2		0.93
CV (%)		7.6
		Trait response mean squares
Source of variation	df	PSE
Replicates	2	0.001
Cultivars	5	0.03**
Error	10	0.001
R^2		0.89
CV (%)		10.2

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.
ANOVA = Analysis of variance. CV = coefficient of variation.
df = degrees of freedom.

Table 3.7. Summary of correlation coefficients values for EL, chlorophyll *a*, chlorophyll *b*, total chlorophyll and PSE with drought susceptibility index (DSI) for six hexaploid wheat cultivars grown under non-drought and drought conditions.

	DSI		
Plant trait	Non-drought	Drought	Response
EL	0.51^{ns}	- 0.77**	- 0.94**
Chlorophyll *a*	0.79**	0.02^{ns}	0.61^{ns}
Chlorophyll *b*	0.38^{ns}	0.29^{ns}	$- 0.37^{ns}$
Total chlorophyll	0.81**	0.24^{ns}	0.43^{ns}
PSE	0.32^{ns}	$- 0.05^{ns}$	0.46^{ns}

Summary of stepwise multiple regression for DSI (dependent variable) and independent traits that showed significant contribution in predicting DSI under control, drought stress, and response.

†(1) Control		
One-variable model	Regression equation	R^2
Total Chlorophyll (X_1)	DSI = - 0.91 + 0.87 X_1	0.66**
†(2) Drought stress		
One-variable model	Regression equation	
EL (X_2)	DSI = 2.09 - 0.05X_2	0.71**
†(3) Responses		
One-variable model	Regression equation	
EL (X_2)	DSI = 1.20 - 0.04 X_2	0.89**

df = degrees of freedom. *, ** Significant at $P < 0.05$ and $P < 0.01$, respectively. (r, $n = 6$).
EL = electrolyte leakage. PSE = photosynthetic efficiency.
DSI = drought susceptibility index.
† EL, chlorophyll *a*, chlorophyll *b*, total chlorophyll and PSE were entered as independent variables in the stepwise multiple regression model.

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CHAPTER 4

DROUGHT-INDUCED CHANGES IN GRAIN YIELD AND YIELD COMPONENTS IN WHEAT CULTIVARS

4.1 INTRODUCTION

Developing high grain yielding cultivars is one of the major objectives of wheat breeding programmes in water-limited environments. Achieving this objective has been relatively slow because of inadequate and impractical physiological screening methods together with the genetic complexity of plant responses to drought (Blum et al., 1981, Blum, 1988). Thus, breeding for drought tolerance has often relied on grain yield and yield components (Edmeades et al., 1989; Yang and Baker, 1991). Drought tolerance can be quantified by the drought susceptibility index (DSI) where cultivars with relatively low DSI are considered tolerant (Fischer and Maurer, 1978). DSI has been used reliably to estimate relative stress injury accounting for variation in yield potential and stress intensity (Bruckner and Frohberg, 1987).

According to Davidson and Chevalier (1990) wheat grain yield is primarily determined by the number of spike-bearing tillers per plant per unit area. The decrease of grain yield caused by drought is partly a result of the reduction in number of tillers that survive and bear kernels (Baker, 1996; Ehdaie and Waines, 1996). Even under sufficient moisture regimes, not all potential tillers may survive let alone develop fertile spikes. The loss of these potential grain bearing tillers may limit sink capacity and thus decrease grain yield. The other yield components that constitute yield are the number of kernels spike^{-1} and kernel weight.

Studies that show which agronomic traits and grain yield components are related to DSI amongst wheat cultivars of varying genetic backgrounds are scarce (Dib et al., 1994). There is a need to establish which yield-related traits respond most to drought and to determine whether any can be used to predict DSI.

Hypothesis. The hypothesis of the present study was that inducing early drought in wheat at the Haun 7.+ growth stage (Haun, 1973) would significantly affect one or more wheat yield-related traits, which could then be used to predict drought tolerance, based on DSI as defined by Fischer and Maurer (1978).

Objectives. The objectives of this study were: (1) to evaluate the effects of early drought, at the Haun 7.+ growth stage (Haun, 1973), on eight wheat cultivars, and (2) to determine which yield-related traits can be used to predict cultivar drought tolerance described by

the DSI. The traits evaluated included grain yield, biomass, thousand kernel weight (TKW), number of kernels spike^{-1}, number of fertile spikes, total number of tillers and plant height.

4.2 MATERIALS AND METHODS

4.2.1 Drought susceptibility index (DSI)

A separate experiment as described in Chapter 2 using a common drought protocol was used to derive the DSI values. The separate experiment was necessary to avoid autocorrelation of grain yield and yield component data with DSI. At 30 DAP and thereafter until physiological maturity, the drought treated plants were watered similar to the non-drought. In the non-drought treatment, water content was maintained at 0.18 kg kg^{-1} dry wt. growing medium throughout the experiment, until physiological maturity. At physiological maturity grain yield was measured and used for computing DSI using the equation [1.1] described in Chapter 1, Section 1.1.7.1 (Fischer and Maurer, 1978).

4.2.2 Cultivars

A drought study was conducted in a growth room at the University of Alberta, Edmonton, Canada. This study used two tetraploid and six hexaploid wheat cultivars. The tetraploids were selected as cultivars of putatively dissimilar drought tolerance based on previous studies, whilst the hexaploid wheat cultivars were also selected to exemplify drought tolerance and a range of genetic diversity, pedigree and morphological differences. The two tetraploid and six hexaploid cultivars are described in detail in Chapter 2, Table 2.1.

Plant growth conditions. The details of the plant growth conditions until 30 days after planting (DAP) are described in Chapter 2. Briefly, drought was induced by withholding water at two intervals. The first interval started at 16 DAP until 20 DAP. The plants were re-watered to field capacity at 20 DAP. The second drought interval was started immediately after 20 DAP by withholding watering until 30 DAP as described in Chapter 2. The growing medium water status was determined gravimetrically by weighing each pot. The water content for the drought treatments was maintained 0.06 kg kg^{-1} growing medium dry weight during the 10-day stress period between 20 and 30 DAP when the plants had developed to the Haun 7.+ growth stage (Haun, 1973). Thereafter, the drought-stressed plants were watered similar to the non-drought. In the non-drought treatment, available water was maintained as described in Section 4.2.1 until physiological maturity.

Sampling. All the samplings in this experiment were conducted after physiological maturity for each treatment. Physiological maturity was defined as the stage of maximum dry

matter accumulation in the kernels and was identified when the peduncle was fully dry. For all traits sampled, four plants per pot (one pot per treatment) were used for measurement of all yield-related traits. Samples for weight measurements were oven dried at 65 °C for 72 h. This was repeated several times until constant weights were obtained and recorded. The traits sampled included plant height (cm), measured from the plant base to the tip of the spike, excluding the awns. The total number of kernels spike^{-1}, tillers and fertile spikes were counted. Grain yield (g), biomass (g), and TKW (g) were measured.

4.2.3 Experimental Design and Statistical Analysis

The experiment was conducted as a split-plot with three replicates comprising two watering treatments as the main-plots and eight cultivars as the sub-plots. Standard analyses of variance were conducted for each of the measured traits using Statistical Analysis System (SAS, 1990) PROC GLM. The Fisher's least significant difference (LSD, $P < 0.05$) was used to separate cultivar means. Correlation coefficients were computed amongst measured traits using PROC CORR. The stepwise multiple regression analyses were performed using the SAS model PROC STEPWISE described in Chapter 2.

4.3 RESULTS

Overall, imposed drought at the Haun 7.+ growth stage (Haun, 1973), caused a reduction in grain yield and each of the assayed yield-related traits, for both the tetraploid and hexaploid wheat cultivars.

4.3.1 Tetraploid Wheat Cultivars

Results for the two tetraploid cultivars (Pelissier and Hercules) in Tables 4.1a and 4.1b show the effects of induced drought at the Haun 7.+ growth stage (Haun, 1973). The ANOVA indicates that there were significant ($P < 0.05$) cultivar, treatment, cultivar x treatment effects, as well as cultivar responses to drought for grain yield, biomass and TKW. Pelissier produced more biomass than Hercules. Hercules had a higher decrease in TKW than Pelissier (Table 4.1a).

Tables 4.2a and 4.2b show results for kernels spike^{-1}, fertile spikes, total tillers and plant height. The ANOVA shows significant cultivar, treatment, cultivar x treatment effects, and cultivar responses to drought for fertile spikes and total tillers. Cultivar, and treatment effects were significant for kernels spike^{-1} fertile spikes, total tillers and plant height. Kernels spike^{-1} were reduced significantly in both cultivars. Pelissier had a lower negative response to drought

relative to Hercules for kernels spike^{-1} (Table 4.2a). Imposed early drought reduced the number of fertile spikes in both cultivars, but Pelissier still produced more fertile spikes than did Hercules.

4.3.2 Hexaploid Wheat Cultivars

The results for grain yield (g), biomass (g), TKW (g), kernels spike^{-1}, fertile tillers, total tillers and plant height are shown in Tables 4.3a - 4.4c. The ANOVA indicates significant cultivar, treatment, cultivar x treatment effects, and response to drought, for all seven traits measured. The lowest negative grain yield response amongst the tested cultivars was shown by K. Nyangumi, whereas Pasa had the highest negative grain yield response to drought. Under drought, K. Mbweha and Pasa showed the lowest biomass, whereas K. Nyangumi had the highest biomass. Kwale had the lowest negative response to drought, whilst Pitic 62 showed the highest negative response to drought.

Table 4.3a indicates that Kwale had the highest TKW under drought conditions and K. Mbweha had the lowest TKW. Table 4.4a indicates that, under non-drought and drought, Pitic 62 and Biggar had the largest kernels spike^{-1} and the other cultivars were similar in kernels spike^{-1}. Pasa had the highest negative response to drought in terms of kernels spike^{-1}, whereas K. Mbweha had the lowest response. Under drought, K. Mbweha had the highest number of fertile spikes, whereas the remaining cultivars had similar numbers of fertile spikes. K. Nyangumi showed the least negative response in number of fertile spikes. Pitic 62, K. Mbweha and Pasa had similar and high negative responses for number of fertile spikes. Biggar and Kwale showed similar and intermediate negative responses to drought.

Under drought conditions, K. Mbweha, K. Nyangumi and Pasa produced the largest number of tillers and Kwale produced the least (Table 4.4a). Pitic 62 had the largest negative response to drought for tiller numbers, whereas Biggar had the smallest response. The imposed drought caused a significant decrease in plant height for the six hexaploid cultivars. For example, Pitic 62, Biggar, and K. Nyangumi had the smallest negative response to drought for plant height, whereas Pasa had the largest response and Kwale and K. Nyangumi had an intermediate response.

Correlations of all seven traits with DSI were nonsignificant, but significant ($P < 0.05$) for kernels spike^{-1} and plant height, under drought conditions. Using the two variables combined in a multiple regression prediction for DSI did not significantly increase the R^2.

4.4 DISCUSSION

Grain yield. Wheat grain yield is a complex trait that responds to interaction amongst many primary genetic factors and the environment. In the present study, the putatively drought

tolerant cultivar Pelissier produced more grain compared to the drought susceptible cultivar Hercules (Table 4.1a). This confirms their relative perfomance in previous field studies (Clarke and Richards, 1988; Hurd, 1974). This shows that conducting grain yield tests under controlled conditions can give results that correlate with tests conducted under field conditions. This approach can be used by wheat breeders who may want to do detailed yield evaluation for sub-groups of cultivars.

The present results agree with Simane et al. (1993) and Van den Boogaard et al (1996) that grain yield is reduced by drought. All cultivars, tolerant and susceptible, were to some degree affected by drought. The present results confirm that an early drought imposed at the Haun 7.+ growth stage (Haun, 1973), can significantly reduce grain yield. It is commonly known from previous studies that grain yield is determined early during plant growth stages (Garcia del Moral et al., 1991). If the grain yield components are developed sequentially, with later-developing components under the non-drought of earlier-developing components it could be possible to apply drought at a given plant growth stage and select for particular yield components.

The current results show that there are cultivars that have relatively large yield potential such as Pasa, but have high negative grain yield responses to drought. In contrast, one can identify a cultivar such as K. Nyangumi that has a low yield potential but a low negative drought response. This has important implications in a wheat breeding programme. It begs the question whether one should breed for cultivars such as Pasa or K. Nyangumi. Incidentally, wheat farmers in Kenya, especially those in the low altitudes where rainfall is critical, prefer to grow K. Nyangumi rather than other cultivars (pers. comm., KARI unpublished data).

There are notable differences for grain yield *per se* and grain yield response to drought amongst the set of wheat cultivars evaluated in the current study. The significant cultivar x treatment interaction for response to drought indicates that there is genetic variability for grain yield response to pre-anthesis drought. However, our results demonstrated non-significant correlation between DSI and grain yield. The lack of correlation between grain yield *per se* and DSI agrees with Clarke et al. (1992) who field-tested 25 hexaploid and 16 tetraploid wheat cultivars. Their results showed that one cultivar can have higher yield than another under dry conditions not because of drought tolerance but, because of higher yield potential under both drought and non-drought conditions.

Biomass. The present results (Table 4.1a) confirm a previous study by Belay et al. (1993) that cultivars can vary in biomass. This is important as high biomass is associated with high grain yield (Ehdaie, 1995).

The findings of the present study agree with those of Van den Boogaard et al. (1996) who demonstrated that biomass is reduced by drought. Inferences can be made that, under drought, there are cultivars, such as Kwale, that are least affected in terms of biomass.

The current results show that both the tetraploid and hexaploid wheat cultivars responded differently to early drought in terms of biomass. However, the results indicate that biomass could not be used to predict DSI, hence the two traits are independent.

TKW. One of the objectives of the current study was to identify yield components that are significantly correlated with DSI. TKW was not significantly correlated with DSI. The current results are in accord with a study by Tesemma et al. (1993) who showed that TKW is adversely affected by drought. The growth of the wheat grain, from initiation to maturity, follows a complex course, of several phases that can be affected by environmental conditions almost until maturity (Evans et al., 1975). Also, the current study agrees with Evans et al. (1975) that seed density, as measured by TKW, decreases when drought is imposed at the Haun 7.+ growth stage (Haun, 1973). In a previous study, Belay et al. (1993) demonstrated that TKW can be used as a predictor for grain yield. In the present study, Pelissier (tolerant) with low DSI compared to Hercules (susceptible) had the lowest TKW response to drought. However, to reach a conclusion that there is a relationship between DSI and TKW will require testing more cultivars in a correlation analysis.

Kernels spike $^{-1}$. The current results support Belay et al. (1993) that kernels spike^{-1} are affected by drought. Their study evaluated 60 tetraploid wheat landraces including the commercial cultivar (Boohai) from the central highlands of Ethiopia. They found that kernels spike^{-1} were a critical determinant of grain yield per unit area. The present results suggest that, under drought conditions, part of the grain yield advantage (e.g., for Pelissier over Hercules) is an attribute of the number of kernels spike^{-1}. Also, Pelissier was observed to be taller than Hercules. A prior study by Pinthus et al. (1983) has shown that, under drought conditions, tall wheat cultivars have higher mean kernels spike^{-1} than short cultivars.

It is expected that drought tolerant tetraploid or hexaploid cultivars should have more kernels spike^{-1} coupled with low DSI. This model fits the two tetraploid cultivars that were tested in the present study. For this suggestion to be acceptable on a wider scale, more tetraploid cultivars should be tested. Also, in previous studies (Hurd, 1974) demonstrated that tall wheat cultivars tend to have extensive root systems for extracting water and nutrients from the environment. Although in the current study roots were not measured, their influence based on previous studies is worth mentioning.

Under drought, DSI had a significant negative correlation with kernels spike^{-1} suggesting that drought tolerant wheat cultivars are those that are capable of maintaining relatively high number of kernels spike^{-1}. Pitic 62 and Biggar (Table 4.4a) produced large number of kernels spike^{-1} and low DSI.

Fertile spikes. The present results agree with Baker (1996) who suggested that under drought the high grain yield of a wheat cultivar is partly an attribute of the number of fertile spikes. This study confirmed that the fertility of a wheat plant can be affected by an early drought at the Haun 7.+ growth stage (Haun, 1973). Perhaps cultivars with relatively low number of fertile tillers have a tendency to abort whole spikes in response to drought.

Total tillers. Results from the present study agree with those of Davidson and Chevalier (1990) who demonstrated that drought reduces the number of surviving tillers. For example, results from the present study show Pelissier has a higher tillering capacity. This corroborates previous studies by Bremner and Rawson (1978) who had described drought tolerant cultivars as those that have both high tiller production and survival capacities. Fraser and Eaton (1983) reported that, under drought conditions, tall wheat cultivars tend to develop and maintain more tiller numbers relative to the short cultivars. The high number of tillers observed in Pelissier (tall cultivar) indicates a potential source of the higher grain yield. Ideally, it is expected that, at various drought levels, drought tolerant wheats should have relatively more fertile tillers and low DSI compared to susceptible cultivars.

The significant cultivar x treatment interaction confirms that the development of tillers is controlled by genetic factors that are sensitive to drought. As noted by Garcia del Moral (1991) the final number of tillers is fixed in small cereals such as wheat by the time of stem elongation. Thus, present results confirm that imposing drought at the Haun 7.+ growth stage (Haun, 1973), as was conducted in this study, can adversely affect tillering capacity of hexaploid wheat cultivars. Selection for total tillers would not be useful for improvement of drought tolerance, as

Plant height. The present results agree with Jana et al. (1990) who indicated that, under drought, the height of tall durum cultivars is not affected as much as short cultivars. The relative influence of plant height on drought tolerance would be particularly important to breeders. However, according to Allan (1989) plant height is a trait that is influenced by height genes (e.g., Rht_1 and Rht_2) that are pleiotropic to other traits such as grain yield and leaf area. Table 4.5 shows correlations amongst traits under both non-drought and drought conditions.

There are many wheat traits that have been associated with drought tolerance. For instance, Belay et al. (1993), who used 60 durum wheats, concluded that under drought conditions tall

cultivars produced more grain than short cultivars. Applying this example to the present study would require more studies, perhaps using near-isogenic lines of Pelissier and Hercules to determine whether height is a factor causing grain yield variation amongst wheat cultivars.

The significant correlation between DSI and plant height in Table 4.5 suggests that tall wheat cultivars are tolerant to drought. Other studies (Ehdai and Waines, 1996; Thompson and Chase, 1992) have demonstrated that the height of a wheat cultivar in relation to grain yield is an important trait to consider especially when testing cultivars under drought conditions.

4.5 Summary and Conclusions

Tetraploids. The results of the present study show that the tetraploid wheat cultivars Pelissier and Hercules are different based on DSI, grain yield, biomass, TKW, kernels spike^{-1}, fertile spikes, total tillers and plant height.

1). The current results demonstrate that the two tetraploid cultivars respond differently to drought for grain yield, biomass, TKW, kernels spike^{-1}, fertile spikes, total tillers and plant height. This controlled growth room study helped to confirm prior studies (e.g., Clarke, 1992; Clarke et al., 1991) that, under drought, these cultivars are distinct in their adaptative response.

2). The present results show that controlled environment studies can give results that parallel those conducted in the field under drought conditions (Clarke and Richards, 1988; Clarke et al., 1991) for two tetraploid wheat cultivars.

Hexaploids. The current results confirm that imposed drought at the Haun 7.+ growth stage (Haun, 1973), can significantly impact the yield-related traits of wheat cultivars measured at physiological maturity.

1). The present results show that, under controlled drought conditions, DSI was significantly correlated with kernels spike^{-1} and plant height. This finding suggests that, under drought conditions, breeders can make gains in drought tolerance by selecting for kernels spike^{-1}, and plant height.

2). The present results demonstrate that it is possible to predict DSI under drought conditions using kernels spike^{-1} and plant height. These results support the conclusion of Ceccarelli et al (1991) that traits such as kernels spike^{-1} and plant height are the key to a breeding programme for drought, but gains are expected to be slow because of the low prediction values.

In conclusion, the current study demonstrated that although all the seven assayed traits were negatively affected by early drought only kernels spike^{-1} and plant height could be used as predictors of DSI. Our study shows that when multiple regression was done, no multiple variable

prediction equations were identifiable that gave better correlations than single variables alone. Based on these observations it was concluded that traits such as kernels spike^{-1} and plant height can be used in wheat breeding programmes for drought tolerance. Also, each trait may require independent consideration.

Table 4.1a Mean ± SE for grain yield, biomass and TKW for tetraploid wheat cultivars grown under non-drought and drought conditions.

	Grain yield (g pot^{-1})[†]			Biomass (g pot^{-1})		
Cultivar	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	19.4 ± 0.6	17.5 ± 0.2	- 1.9 ± 0.6	57.5 ± 0.5	49.2 ± 0.3	- 8.4 ± 0.3
Hercules	14.9 ± 0.3	9.4 ± 0.3	- 5.6 ± 0.4	42.9 ± 0.3	30.3 ± 0.2	- 12.6 ± 0.4
Mean	17.2 ± 0.6	13.5 ± 0.9		50.2 ± 0.4	39.8 ± 0.2	

	Thousand kernel weight (TKW) (g)		
[†]Cultivar	Non-drought	Drought	Response
Pelissier	47.1 ± 0.1	45.3 ± 0.3	- 1.8 ± 0.3
Hercules	47.4 ± 0.1	44.8 ± 0.2	- 2.6 ± 0.1
Mean	47.3 ± 0.1	45.1 ± 0.2	

[†] Every pot contained 4 plants.

Table 4.1b. Summary of ANOVA for grain yield, biomass and thousand kernel weight (TKW) for two tetraploid wheat cultivars grown under non-drought and drought conditions.

Source of variation	df	Trait mean squares: Grain yield (g pot^{-1})†	Biomass (g pot^{-1})	TKW (g)
Replicates	2	0.1	5.3	0.1
Cultivars (C)	1	116.1**	72.3*	0.4*
Error (*a*)	2	0.8	3.1	0.02
Treatments (T)	1	41.6**	540.3**	3.3**
C x T	1	10.3**	283.8**	1.2**
Error (*b*)	4	0.3	8.6	0.02
R^2		0.96	0.93	0.96
CV (%)		4.0	6.8	3.3

Source of variation	df	Trait response mean squares: Grain yield (g pot^{-1})	Biomass (g pot^{-1})	TKW (g)
Replicates	2	0.8	12.8	0.01
Cultivars	1	20.6*	567.6*	0.84*
Error	2	0.7	21.6	0.02
R^2		0.76	0.75	0.78
CV (%)		23.1	24.7	21.6

df = Degrees of freedom.
CV = Coefficient of variation.
*, ** Significant *F*-test at $P < 0.05$ and $P < 0.01$, respectively.
† Every pot had 4 plants.

Table 4.2a. Mean ± SE values for kernels spike^{-1}, fertile spikes, total tillers and plant height for two tetraploid wheat cultivars grown under non-drought and drought conditions.

	Kernels spike^{-1}			Fertile spikes pot^{-1}		
Cultivar†	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	37.4 ± 1.1	31.6 ± 0.4	- 5.8 ± 0.9	13.7 ± 0.7	12.0 ± 0.6	- 1.9 ± 0.5
Hercules	29.0 ± 1.3	21.6 ± 1.1	- 7.4 ± 1.2	13.0 ± 0.6	8.3 ± 0.7	- 4.3 ± 0.3
Mean	33.2 ± 1.2	26.6 ± 0.4		13.4 ± 0.8	10.2 ± 0.6	

	Total tillers pot^{-1}			Plant height (cm)		
Cultivar†	Non-drought	Drought	Response	Non-drought	Drought	Response
Pelissier	30.3 ± 1.2	18.0 ± 2.3	- 12.3 ± 1.2	99.0 ± 0.4	91.3 ± 1.3	- 7.7 ± 0.8
Hercules	25.3 ± 2.2	8.6 ± 0.3	- 17.2 ± 1.4	83.3 ± 0.8	74.7 ± 0.9	- 8.6 ± 1.1
Mean	27.3 ± 1.4	13.3 ± 1.3		91.2 ± 1.5	81.5 ± 1.3	

Table 4.2b. Summary of ANOVA for kernels spike^{-1}, fertile spikes, total tillers and plant height for two tetraploid wheat grown under non-drought and drought conditions.

		Trait mean squares			
Source of variation	df	Kernels spike^{-1}	Fertile spikes	Total tillers	Plant height (cm)
Replicates	2	7.1	1.6	3.3	36.1
Cultivars (C)	1	253.9**	12.0**	102.1**	784.1**
Error (*a*)	2	2.3	0.6	3.1	20.2
Treatments (T)	1	129.4**	27.0**	520.2**	200.1**
C x T	1	1.9ns	5.3*	114.1**	18.8^{ns}
Error (*b*)	4	1.5	0.2	4.3	10.1
R^2		0.94	0.91	0.91	0.94
CV (%)		4.2	9.1	8.4	5.2

		Trait response mean squares			
Source of variation	df	Kernels spike^{-1}	Fertile spikes	Total tillers	Plant height
Replicates	2	3.8	1.2	15.2	1.5
Cultivars	1	2.8^{ns}	8.2*	228.2**	5.1^{ns}
Error	2	0.5	0.2	2.2	3.5
R^2		0.89	0.84	0.88	0.82
CV (%)		11.2	16.4	11.2	17.6

df = Degrees of freedom.
CV = Coefficient of variation.
*, ** Significant *F*-test at $P < 0.05$ and $P < 0.01$, respectively.
^{ns} Not significant.

Table 4.3a. Mean values for grain yield, biomass and TKW for six hexaploid wheat cultivars grown under non-drought and drought conditions.

Cultivar[†]	Grain yield (g pot^{-1})[¶]			Biomass (g pot^{-1})		
	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	27.8 b[‡]	16.8 ab	- 10.9 abc	71.0 a	44.1 b	- 26.9 a
Biggar	29.9 ab	16.1 ab	- 13.8 ab	68.1 ab	46.3 b	- 21.7 ab
Kwale	28.2 ab	16.0 ab	- 12.1 abc	57.0 cd	48.3 b	- 8.6 c
K. Mbweha	21.3 c	13.1 b	- 8.2 bc	54.6 d	35.4 c	- 19.2 ab
K. Nyangumi	17.9 c	11.9 c	- 5.9 d	72.1 a	55.6 a	- 16.5 b
Pasa	33.8 a	18.9 a	- 14.9 a	62.6 bc	35.8 c	- 26.8 a
Mean	26.5 ± 2.3	15.5 ± 1.0		64.2 ± 2.0	44.3 ± 2.2	

Cultivar	Thousand kernel weight (TKW) (g)		
	Non-drought	Drought	Response
Pitic 62	45.4 b	41.7 bc	- 3.7 bc
Biggar	49.6 ab	43.8 ab	- 5.8 b
Kwale	50.7 ab	48.9 a	- 1.8 c
K. Mbweha	38.9 c	36.8 c	- 2.1 c
K. Nyangumi	47.4 b	43.0 ab	- 4.4 bc
Pasa	54.7 a	42.4 b	- 12.3 a
Mean	47.8 ± 1.1	42.6 ± 1.7	

[†] Cultivars are listed in the order of DSI in Chapter 2, Table 2.3a.
[‡] Means within the same column followed by the same letter are not significantly different at $P < 0.05$, according to least significant difference comparison.
[¶] Every pot had 4 plants.

Table 4.3b. Summary of ANOVA for grain yield, biomass and thousand kernel weight (TKW) for six hexaploid wheat grown under non-drought and drought conditions.

Source of variation	df	Trait mean squares		
		Grain yield ($g\ pot^{-1}$)‡	Biomass ($g\ pot^{-1}$)‡	TKW (g)
Replicates	2	9.2	19.8	0.2
Cultivars (C)	5	104.7**	270.2**	1.2**
Error (*a*)	10	0.8	6.2	0.02
Treatments (T)	1	1092.4**	3588.6**	2.4**
C x T	5	17.2**	71.4**	0.2*
Error (*b*)	12	0.4	3.6	0.01
R^2		0.84	0.93	0.92
CV (%)		15.5	6.8	7.2

Source of variation	df	Trait responses mean squares		
		Grain yield ($g\ pot^{-1}$)‡	Biomass ($g\ pot^{-1}$)‡	TKW (g)
Replicates	2	6.0	2.7	0.1
Cultivars	5	34.4*	142.8**	0.4*
Error	10	12.3	18.3	0.1
R^2		0.88	0.78	0.81
CV (%)		12.1	21.4	19.2

df = Degrees of freedom.
CV = Coefficient of variation.
*, ** Significant *F*-test at $P < 0.05$ and $P < 0.01$, respectively.

Table 4.4a. Mean values for kernels spike^{-1}, fertile spikes, total tillers and plant height for six hexaploid wheat cultivars grown under non-drought and drought conditions.

	Kernels spike^{-1}			Fertile spikes pot^{-1}		
Cultivar[†]	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	51.5 a[‡]	48.1 a	- 3.3 c	19.6 bc	9.3 b	- 10.3 a
Biggar	53.5 a	47.7 a	- 5.7 b	17.6 bc	9.9 b	- 7.7 b
Kwale	38.7 c	32.4 b	- 6.4 b	16.6 c	9.3 b	- 7.3 b
K. Mbweha	35.6 c	33.2 b	- 2.4 c	27.0 a	15.3 a	- 11.6 a
K. Nyangumi	39.0 c	33.6 b	- 5.3 b	13.0 d	9.3 b	- 3.7 c
Pasa	45.7 b	33.1 b	- 12.6 a	20.3 c	10.6 b	- 9.6 a
Mean	44.0 ± 1.0	38.0 ± 1.1		19.0 ± 1.1	10.6 ± 0.9	

	Total tillers pot^{-1}			Plant height (cm)		
Cultivar[†]	Non-drought	Drought	Response	Non-drought	Drought	Response
Pitic 62	28.3 a[‡]	17.6 b	- 10.6 a	87.3 a	79.0 a	- 8.3 b
Biggar	20.6 bc	18.0 b	- 2.6 c	70.6 c	65.6 bc	- 5.0 c
Kwale	18.3 c	13.7.c	- 4.6 b	75.6 b	67.3 b	- 8.3 b
K. Mbweha	28.6 a	23.3 a	- 5.3 b	63.3 d	54.3 e	- 8.9 b
K. Nyangumi	27.6 a	23.6 a	- 4.0 b	64.6 d	59.3 d	- 5.3 c
Pasa	22.3 b	17.6 b	- 4.7 b	76.3 b	62.3 c	- 14.0 a
Means	24.3 ± 1.8	18.9 ± 1.2		72.9 ± 1.6	64.6 ± 1.4	

[†] Cultivars are listed in the order of DSI in Chapter 2, Table 2.3a.

[‡] Means within the same column followed by the same letter are not significant different at $P < 0.05$, according to least significant difference comparison.

Table 4.4b. Summary of ANOVA for kernels spike^{-1}, fertile spikes, total tillers and plant height for six hexaploid wheat grown under non-drought and drought conditions.

		Trait mean squares			
Source of variation	df	Kernels spike^{-1}	Fertile spikes pot^{-1}	Total tillers pot^{-1}	Plant height (cm)
Replicates	2	3.5	0.2	6.0	5.3
Cultivars (C)	5	321.5**	71.1**	67.5**	431.9**
Error (*a*)	10	13.4	2.6	4.2	8.7
Treatments (T)	1	321.0**	650.3**	191.4**	625.0**
C x T	5	19.5**	11.9*	17.9**	15.8*
Error (*b*)	12	3.4	2.4	1.9	2.6
R^2		0.94	0.86	0.94	0.94
CV (%)		5.1	12.5	6.2	5.8

		Trait response mean squares			
Source of variation	df	Kernels spike^{-1}	Fertile spikes pot^{-1}	Total tillers pot^{-1}	Plant height (cm)
Replicates	2	3.9	10.2	0.7	3.6
Cultivars	5	38.9*	23.7*	31.2**	48.6**
Error	10	9.8	6.1	3.9	9.1
R^2		0.78	0.70	0.78	0.75
CV (%)		22.5	29.2	21.8	24.9

df = Degrees of freedom.
CV = Coefficient of variation.
*, ** Significant *F*-test at $P < 0.05$ and $P < 0.01$.

Table 4.5. Simple correlation coefficients between pairs of traits for six hexaploid wheat cultivars grown under non-drought conditions and drought conditions

Plant trait	DSI		
	Non-drought	Drought	Response
Grain yield	- 0.17 ^{ns}	- 0.18 ^{ns}	- 0.11 ^{ns}
Biomass	- 0.37 ^{ns}	- 0.26 ^{ns}	- 0.13 ^{ns}
TKW	0.15 ^{ns}	0.23 ^{ns}	0.50 ^{ns}
Kernels spike^{-1}	- 0.62 ^{ns}	- 0.84 **	0.60 ^{ns}
Fertile spikes	0.13 ^{ns}	0.40 ^{ns}	- 0.25 ^{ns}
Total tillers	0.02 ^{ns}	0.42 ^{ns}	- 0.59 ^{ns}
Plant height	- 0.63 ^{ns}	- 0.84 **	- 0.43 ^{ns}

Summary of stepwise multiple regression for DSI (dependent variable) and traits that were loaded as the significant predictors for DSI under control and drought stress with response.

	†(1) Control	
One-variable model	Regression equation	R^2
Grains spike^{-1} (X_1)	DSI = - 0.70 - 0.01X_1	0.12

	†(2) Drought stress	
Two-variable model	Regression equation	
Total tillers (X_2)	DSI = - 0.48 + 0.06 X_2	0.14
Fertile spikes (X_3)	DSI = - 0.48 + 0.06 X_2 + -0.05 X_3	0.31

	†(3) Responses	
One-variable model	Regression equation	
Fertile spikes (X_4)	DSI = 1.8 - 0.03 X_4	0.27
Two-variable model		
Fertile spikes (X_4)		
Plant height (X_5)	DSI = 1.4 - 0.04 X_4 + 0.06X_5	0.36

*, ** Significant F-value (r, $n = 6$) at $P < 0.05$ and $P < 0.01$, respectively.

^{ns} Not significant, TKW = Thousand kernel weight. DSI = drought susceptibility index.

†Thousand kernel weight, grains spike^{-1}, fertile spikes, total number of tillers and plant height were entered as independent variables in the stepwise multiple regression model.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1 Background

Development of drought-tolerant wheat cultivars targeted for drought-prone regions is imperative. However, success has been limited partly by inadequate screening methods and lack of genotypes that show clear contrasts in response to well-defined environments (Ludlow, 1989).

Wheat breeders have long known that there are certain cultivars that respond well over a wide range of environments. Just how or why such 'universal' cultivars maintain their relative standing compared to other cultivars is a most intriguing question. The universally adapted cultivars must either tolerate change or adjust favourably to changes in environment. Characteristics of known tolerant and nontolerant cultivars can lead to identification of improved selection techniques for drought tolerance.

The present study focussed on drought tolerance that was defined on the basis of a DSI. Various other assays were conducted after subjecting wheat cultivars to drought under controlled environment conditions at the Haun 7.+ growth stage (Haun, 1973). Throughout this study, it was evident that imposed drought at the Haun 7.+ growth stage had differential responses both on the two tetraploid cultivars Pelissier and Hercules, as well as amongst the six hexaploid cultivars Pitic 62, Biggar, Kwale, K. Mbweha, K. Nyangumi and Pasa.

5.2 Retrospect

Objectives. The intent of the present study was to: (1) to assess the effects of drought on eight wheat cultivars (two tetraploid and six hexaploid) of diverse genetic background and reported drought tolerance level. This required measuring the responses of the traits to imposed drought conditions, using many parameters, including relative water content (RWC), electrolyte leakage (EL), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl T), chlorophyll fluorescence(Chl F), sucrose, glucose, fructose, fructan, total free amino acids (TFAA), free proline, nitrate reductase activity, total SDS-soluble proteins (TSP), grain yield and grain yield components, and DSI under controlled environment, and (2) to determine the relationships between the listed traits and assess whether these traits can be used effectively for selecting drought-tolerant wheat cultivars based on the Fischer and Maurer (1978) drought susceptibility index (DSI).

The fundamental questions in this study are: (1) Can drought at the Haun 7.+ growth stage cause differential effects on the eight selected wheat cultivars? (2) If various assays are conducted simultaneously at 30 DAP (days after planting) after an early drought, can combinations of such assays better predict DSI?, and (3) How will drought at the tillering stage affect grain yield and grain yield components, and which grain yield components would predict DSI?

5.3 Tetraploid Wheat Cultivars

The effects of imposed drought on DSI at the Haun 7.+ growth stage on the two tetraploid wheat cultivars indicated that Pelissier is more drought-tolerant than Hercules. This confirmed previous reports of Clarke and Richards (1988) and Clarke et al. (1991) for these cultivars. Imposed drought increased EL, but the increase was similar in both cultivars. The ability of this growthroom trial to confirm the published reports from field trials is of note, and improves the confidence level in other results from the growthroom trial.

Sucrose, glucose, fructose, fructan, total free amino acids, free proline, TSP and NRA changed differentially in response to drought. The study also found that early imposed drought caused an increase of sucrose for Pelissier but decrease for Hercules. Glucose levels decreased in both cultivars, whereas the levels of fructose, fructan, total free amino acids, proline, total soluble proteins and NRA increased after drought. This research supports previous findings (Blum, 1989; Morgan, 1991) that have demonstrated solute accumulation in wheat cultivars after drought. The significant cultivar interactions with drought level for sucrose, glucose, fructose, total free amino acids and NRA indicate that there are genetic differences between the cultivars for these traits.

Imposed drought at the Haun 7.+ growth stage under controlled environment conditions caused a significant decrease in chlorophyll *a* (Chl *a*) and total chlorophyll (Chl T), but had little effect on Chl *b*. Also, the imposed drought decreased photosynthetic efficiency (PSE). The differential reduction of chlorophyll content and Chl F indicates that the two cultivars have different responses to drought.

There were significant cultivar, cultivar x treatment effects and responses for grain yield, biomass, HI, TKW, kernels spike^{-1}, fertile spikes pot $^{-1}$, total tillers pot $^{-1}$ and plant height. This agrees with prior literature that the occurrence of drought as early as the Haun 7.+ growth stage in a wheat crop can significantly affect traits assayed at maturity (Simane et al., 1993).

5.3.1 Novel Findings

The findings of the present study for the tetraploid cultivars are summarised in Table 5.0.

1). The current findings show that the two tetraploid cultivars differed in terms of total solutes under drought. Pelissier accumulated more solutes (most of the solutes as result of TFAA and TSP) than did Hercules. Wheat cultivars that accumulate relatively high amount of solutes during drought conditions have a relatively higher grain yield potential than cultivars that accumulate relatively low amount of solutes (Morgan, 1984).

2). Pelissier has low NRA. This implies that the cultivar is more efficient in nitrate assimilation relative to Hercules. The relatively high TFAA and TSP, both products of nitrate assimilation, also indicate that Pelissier is a better nitrate assimilator. There is no prior literature that has described these two cultivars in terms of NRA. Prior literature on different cultivars indicates that cultivars with relatively low NRA are also efficient in utilising nitrogen under various environments, including drought conditions (Gallagher et al., 1980).

3). There is no prior literature published about EL, Chl content and PSE response to drought conditions, for Pelissier and Hercules. Under drought conditions, Pelissier with relatively low Chl content had a higher PSE. This indicates that cultivars with relatively high Chl content are likely to be relatively low in photosynthetic efficiency under drought conditions. This finding is important for wheat breeders. It suggests that when selecting for drought tolerance, light green cultivars/lines might be the most suitable for drought conditions. However, more research will be required using a larger number of tetraploid cultivars and segregating populations to determine whether there is a strong enough association between Chl content and DSI justifying using Chl content as a selection tool.

5.4 Hexaploid Wheat Cultivars

The current results demonstrate that the six hexaploid cultivars differ in their carbohydrate response to drought. Drought at the Haun 7.+ growth stage affected levels of sucrose, glucose, fructose, fructan, total amino acids, free proline, total soluble proteins and nitrate reductase activity (NRA). The levels of sucrose, fructose and fructan increased with drought, whereas glucose levels decreased. The levels of total free amino acids, free proline, total soluble proteins and NRA increased with drought. However, the current study failed to find significant correlations between DSI and any of the assayed traits, alone or in combination. McCaig and Clarke (1982) reported that drought increases the level of carbohydrates in wheat leaves by decreasing the demand of sugars by the growing tissues. This may partially explain the increase of sucrose, fructose and fructan for the hexaploid cultivars. Alternatively, increased leaf carbohydrate concentrations appear to be vital in osmoregulation of stressed leaves (Blum, 1989).

5.4.1 Novel Findings

The findings of the current study for the hexaploid cultivars are summarised in Table 5.1.

1). There are no prior published reports on the effects of drought on the Kenyan cultivars Kwale, K. Mbweha, K. Nyangumi and Pasa. This information is important for wheat breeders as it can help in selecting parents for making desired crosses. Rigorous evaluation of potential parental cultivars is vital in wheat breeding.

2). There is no prior published literature on the six hexaploid cultivars regarding Chl content, Chl F, PSE and EL. Also, there are no prior drought studies that have reported the effects of drought on the present six hexaploid cultivars in terms of sucrose, glucose, fructose, fructan, total free amino acids, free proline, total SDS-soluble proteins and nitrate reductase activity, and none in relation to DSI.

For all six cultivars there was an increase in EL. The cultivar x treatment interaction for EL suggests that there are genetic differences in response to drought amongst the tested cultivars. This information is important for wheat breeders as it can help in selecting parents for making desired crosses. However, more research is needed to confirm the heritability of EL.

The current results show that imposed drought at the Haun 7.+ growth stage caused significant decrease in Chl *a* and Chl T, but little effect on Chl *b*. The significant cultivar x treatment interaction for Chl *a* and Chl T indicates that differential response of these traits is genetically influenced, thus they can be manipulated in a breeding program. The correlation results indicate a strong association between DSI and Chl *a* and Chl T under non-drought conditions. Under drought conditions only EL had a significant negative correlation with DSI.

The significant negative correlation between DSI and EL, indicating that cultivars with high EL under drought have a lower DSI, contrasts markedly with the study of Blum and Ebercon (1981) that showed that low EL was correlated with cultivar tolerance to drought. However, in the Blum and Ebercorn (1981) study, heat or high temperature was a factor, whereas in the present study temperature was not a critical factor. The current results are in accord with Morgan (1991) who demonstrated that a significant amount of solute increase during a drought period is the result of osmotic adjustment (OA). Further research would be needed to determine whether solutes measured as EL are in part a consequence of OA, in this particular set of wheat cultivars.

There are reports (e.g., Clarke et al., 1991; McCaig and Romagosa, 1991) that indicate physiological traits cannot fully explain drought tolerance. The present study, which used only one drought level (0.06 kg water kg^{-1} dry weight growing medium) found, for example, that there was no association in the hexaploid wheats between many of the physiological traits and DSI.

There are other drought thresholds that have not been tested and the relationship might change at a different drought threshold. It should also be noted that the study also only used one plant growth stage at Haun 7.+ scale (Haun, 1973) for stress application. Although the Haun 7.+ growth stage was chosen as the critical stage, based on prior literature there are other growth stages that might give different results, e.g., anthesis or post-anthesis (Hay and Kirby, 1991). A combination of various growth stages and levels of drought may change the way a cultivar responds to drought.

5.5 Conclusions

1). The results of the entire study point out that drought imposed at Haun 7.+ growth stage on both tetraploid and hexaploid wheat cultivars affected most of the traits researched. Cultivars responded differentially to drought for these traits. The effects of drought at the Haun 7.+ growth stage also had a significant effect on yield-related traits at physiological maturity. The imposed drought caused significant differences in DSI amongst these eight wheat cultivars of diverse genetic background, selected to represent a range of drought-tolerant potential, and that these differences could be effectively identified under controlled growth room conditions.

2). An unexpected finding was that association of many of the traits with DSI was non-significant in a great many cases, whether assayed under control or droughted conditions, or when the response of the trait to drought was assessed. Many of the traits described in the literature as useful for predicting drought tolerance were not correlated with DSI in this study, notably proline and Chl F. The absence of such relationships for many of the individual traits largely removed the possibility of determining whether use of these variables in combinations would improve predictability for DSI.

3). The study demonstrated, however, that EL, Chl *a*, Chl T, total number of tillers, fertile spikes and plant height could be used effectively to predict a significant amount of the variance in drought tolerance amongst the cultivars considered, based on DSI. Grain yield is the result of many interacting processes, so it is difficult to relate any one trait to grain yield or productivity particularly in the case of different, unrelated cultivars under varied environmental conditions.

4). The current results endorsed the view that the traditional plant breeding protocols of using grain yield and grain yield components may not be completely substituted by physiological predictor trait assays that can specifically determine drought tolerance. However, traits such as chlorophyll content and EL merit further investigation to determine if they would be useful as

indirect selection criteria in breeding of wheat cultivars for dry land cultivation, especially in the case where these could be used effectively under non-drought conditions.

Table 5.0. †Summary of drought study findings compared with what is known in literature for the two tetraploid cultivars Pelissier (tolerant = T) and Hercules (susceptible = S)

Literature source	Trait	Prior studies		‡Present study		Comment
		Tolerant	Susceptible	Tolerant	Susceptible	
	Sucrose	-	-	Low	High	New
	Glucose	-	-	Low	High	New
	Fructose	-	-	High	Low	New
	Fructan	-	-	Low	High	New
	TFAA	-	-	High	Low	New
	Free proline	-	-	Same	Same	New
	TSP	-	-	High	Low	New
	NRA	-	-	Low	High	New
Clarke et al. (1992)	RWC	High	Low	High	Low	Agree
	EL (Drought)	-	-	Low	High	New
	Chl *a*	-	-	Low	High	New
	Chl *b* (Drought)	-	-	Low	High	New
	Chl T	-	-	Low	High	New
	PSE	-	-	High	Low	New
	Biomass	-	-	High	Low	Expected
	HI	-	-	High	Low	Expected
	TKW	-	-	Same	Same	Expected
	Grains spike^{-1}	-	-	High	Low	Expected
	Fertile spikes	-	-	High	Low	Expected

†Comparisons/values in this table are for drought conditions.

Table 5.0 continued.....

Literature source	Trait	Prior study		‡Present study		Comment
		Tolerant	Susceptible	Tolerant	Susceptible	
Hurd (1971)	Total tillers	High	Low	High	Low	Agree
	Height	High	Low	High	Low	Agree
Clarke (1992)	3-year mean days to head	Low	High	-	-	NMIPS
	3-year mean leaf RWL	Low	High	-	-	NMIPS
Clarke et al. (1991)	Transpiration	Low	High	-	-	NMIPS
Hurd (1971)	Total roots	High	Low	-	-	NMIPS
Clarke et al. (1992)	4-year mean grain yield	High	Low	High	Low	Agree
	4-year mean grain yield DSI	Low	High	Low	High	Agree

- = Indicates trait no published information on the cultivar for this trait

‡ = The present study conducted under growth room

NMIPS = Not measured in the present study

Tolerant = Pelissier, and Susceptible = Hercules

TFAA = Total free amino acids

TSP = Total SDS-soluble proteins

NRA = Nitrate reductase activity

Chl *a* = Chlorophyll *a*

Chl *b* = Chlorophyll *b*

Chl T = Total chlorophyll

RWC = Relative water content

EL = Electrolyte leakage

PSE = Photosynthetic efficiency

TKW = Thousand kernel weight

RWL = Relative water loss

Table 5.1. ¶Summary of findings for hexaploid cultivars. All traits are reported under drought conditions except where specified

Literature source	Trait	†Prior study Correlation with DSI	‡ Present study Correlation with DSI	Comments
	Sucrose	-	ns	New
	Glucose	-	ns	New
	Fructose	-	ns	New
	Fructan	-	ns	New
	TFAA	-	ns	New
Dib et al. (1994)	Free proline	** (- ve)	ns	Disagree
	TSP	-	ns	New
	NRA	-	ns	New
	RWC	-	ns	New
	EL (Control)	-	* (+ ve)	New
	EL	-	** (- ve)	New
	EL (Response)	-	** (- ve)	New
	Chl *a* (Control)	-	** (+ ve)	New
	Chl *b*	-	ns	New
	Chl T (Control)	-	** (+ ve)	New
Dib et al. (1994)	PSE	** (- ve)	ns	Disagree
	Grain yield	** (- ve)	ns	Disagree
	Biomass	** (- ve)	ns	Disagree
	TKW	* (- ve)	ns	Disagree
	Grains spike^{-1}	-	** (- ve)	New
	Fertile spikes	-	ns	New
	Total tillers	-	ns	New
	Height	-	** (- ve)	New

Table 5.1. Continued .....

‡ = DSI and other traits in this study assessed in a growth room

† = In prior studies proline was assessed in the growth room, and other traits in the field

¶Comparison/values in this table are for drought conditions except where specified.
(- ve) = negative correlation

(+ ve) = positive correlation

- = Indicates trait not published or no information available

Chl *a* = Chlorophyll *a*

Chl *b* = Chlorophyll *b*

Chl T = Total chlorophyll

TFAA = Total free amino acids

RWC = Relative water content

NRA = Nitrate reductase activity

PSE = Photosynthetic efficiency

TKW = Thousand kernel weight

RWL = Relative water loss

EL = Electrolyte leakage

TSP = Total SDS-soluble proteins

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